ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2016, Vol. 466, pp. 66–69. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © P.D. Kim, S.S. Zamay, T.N. Zamay, V.S. Prokopenko, O.S. Kolovskaya, G.S. Zamay, V.Ya. Princ, V.A. Seleznev, A.I. Komonov, E.A. Spivak, R.Yu. Rudenko, A.V. Dubinina, A.V. Komarov, V.V. Denisenko, M.A. Komarova, A.E. Sokolov, A.A. Narodov, V.P. Zjivaev, A.S. Zamay, 2016, published in Doklady Akademii Nauk, 2016, Vol. 466, No. 5, pp. 616–619.

The Antitumor Effect of Magnetic Nanodisks and DNA Aptamer Conjugates

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Presented by Academician V.F. Shabanov January 19, 2015

Received May 25, 2015

Abstract—Here we describe a method of forming large arrays (up to 10^9 pieces) of free magnetic Ni-nanodisks 50 nm thick coated on both sides with layers of 5 nm thick Au. The antitumor effect of the magnetic nickel gold-coated nanodisks and DNA aptamer conjugates was evaluated in vivo and in vitro. Under the influence of rotating magnetic field, the studied nanodisks can cause the death of Ehrlich ascites carcinoma cells.

DOI: 10.1134/S1607672916010154

Technologies based on using magnetic nanoparticles are actively used in medical and biological research due to their unique optical, electrical, and magnetic properties [1]. Magnetic field-controlled particles are used for both targeted drug delivery and mechanical destruction of target cells. The main directions in the biomedical research with the use of magnetic nanoparticles are the induction of apoptosis in cancer cells, the analysis of substances in biological fluids and tissues, and cell sorting [2–4]. The most promising materials for medical research in cell surgery are nanoparticles with a small residual magnetization. The prospects of using permalloy nanodisks with a vortical magnetization structure for nanosurgery were first shown in brain glioma cell culture [5].

This paper describes the anticancer properties of nickel nanodisks with a dipole magnetization structure, which were exposed to an alternating magnetic field and modified with biorecognizing molecules. The latter were represented by DNA aptamers. Aptamers are single-stranded RNA or DNA fragments forming three-dimensional structures during the interaction of complementary chain fragments, which, due to the unique conformation, can bind to targets specific to the given aptamer [6]. The aim of our experiments was to determine the prospects for using nanodisks modified with DNA aptamers for targeted cell surgery of cancer. For the binding of magnetic disks to ascites cells, we used DNA aptamers (AS9), the structure and function of which was described in [7].

The procedure of forming large arrays of free magnetic Ni-nanodisks 50 nm thick coated on both sides with Au layers 5 nm thick included the following steps: formation of a polymer layer 300 nm thick on a substrate; separation of the polymer layer into islands 500 nm in diameter using a stamp; sequential sputtering of Au–Ni–Au layers on the islands; and liberation of Au–Ni–Au-sputtered disks by dissolving the polymer islands and their subsequent transfer into acetone. Pressing was carried out using an Eitre 6 nanolithography device (Obducat AB, Sweden) using a stamp that yielded up to 10^9 islands on a substrate area of 30 cm^2. The technology included the optimization of the conditions of sputtering, forming, and dissolving the polymer islands. To measure the size and magnetic proper-

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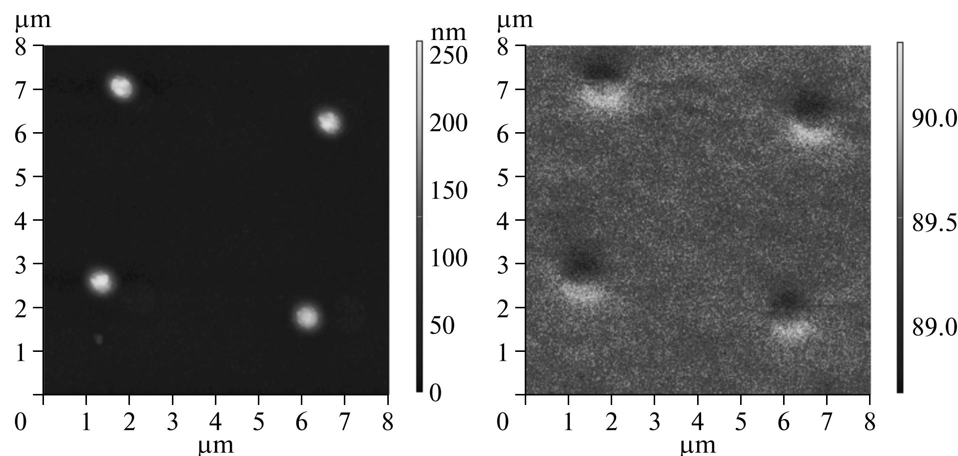

Fig. 1. Results of study of the properties of magnetic disks using atomic force microscopy. The location and size of disks on the polymer islands is shown on the left, and the magnetic dipoles of the same disks formed after their magnetization in a magnetic field of 0.5 T is shown on the right.

ties of the nanodisks, we used a Solver P47-PRO scanning atomic force microscope (NT-MDT, Russia) equipped with magnetic cantilevers. In Fig. 1, the bright areas (on the left) correspond to nanodisks disposed on polymeric islands; magnetic dipoles of the disks formed after their magnetization in the plane parallel to the disks in a magnetic field with induction of 0.5 T are shown on the right. The minimum value of the field at which the nanodisks were remagnetized was 0.3 T.

Magnetic nanodisks separated from the substrate were washed three times in Dulbecco's modified phosphate buffered saline (DPBS) by centrifugation at 15 000*g* for 15 min and then modified with aptamers. For this purpose, primers with thiol groups were incubated with the DNA aptamers, part of which contained the fluorescent label FITC in equimolar concentrations (300 nM), for 18 h at 4°C to obtain hybrids. Next, the solution was supplemented with 30 mM Tris-$HClO_4$ buffer (pH 8.6) in a ratio of Tris-$HClO_4$ buffer : DNA hybrid equal to 1 : 3, mixed with magnetic nanodisks (10^6 hybrid molecules per disk), and incubated at 4°C for 24 h. After incubation, the nanodisks with aptamers immobilized on their surface were washed with DPBS buffer.

Experiments were performed with male outbred ICR mice obtained from the animal breeding facility of the SSC Vector (village Koltsovo, Novosibirsk oblast). Transplantable Ehrlich ascites carcinoma cells were used as a study object. To induce cell binding to the disks, 20 μL of the DPBS buffer containing 10 thousand ascites cells was supplemented with 5 μL of the DPBS buffer containing 50 thousand modified nanodisks (five disks per cell). After 30 min of incubation, required for the binding of the nanodisks to ascites cells, the tubes with cells were placed for 20 min in a rotating magnetic field. After 90 min of exposure, the functional state of ascites cells was estimated using a FluoView FV10i confocal microscope (Olympus, Japan). Four samples of ascites cells were used as a control: cells that were exposed to the magnetic field for 20 min; cells with unmodified magnetic disks; cells with DNA aptamers that were exposed to the magnetic field for 20 min; and cells with immobilized nanodisks that were modified with the DNA aptamers in a ratio of 1 : 5 (cells : nanodisks) without exposure to the magnetic field. The functional state of ascites cells in the control samples was estimated using a FluoView FV10i confocal microscope. The control and experimental samples were studied in three experiments.

Using the scanning atomic force microscopy, we found that the magnetic moment was parallel to the disk plane (Fig. 1). Calculations showed that the magnitude of the magnetic moment of the nanodisk was approximately 10–15 A m^2 and did not change under the influence of a weak external magnetic field (EMF). In the case of EMF induction of 0.01 T, the moment of the forces that act on the disk is approximately 10^{-17} N m. The disk attached to the cell membrane is affected by the elastic forces. The moment of these forces is less than 10^{-18} N m and cannot prevent the rotation of the disk in the EMF direction. Severe stretching of the membrane leads to its damage. In our experiments, the magnetic nanodisks modified with the DNA aptamers triggered cell death in the ascites cell culture, whereas in control samples no death of ascites cells was observed (Fig. 2).

To investigate the anticancer effect of the magnetic nanodisks in vivo, mice with Ehrlich ascites carcinoma were injected into the peritoneal cavity with 300 μL of DPBS buffer containing 10 million nanodisks modified with the DNA aptamers. The control mice were injected with 300 μL of DPBS buffer. Then, 30 min after injection, mice were placed in a unit that generated a rotating magnetic field of 0.01 T. The duration of exposure to the EMF was 20 min. Thereafter, 60 min after exposure to VMP, ascites cells were taken from the experimental and control animals. The

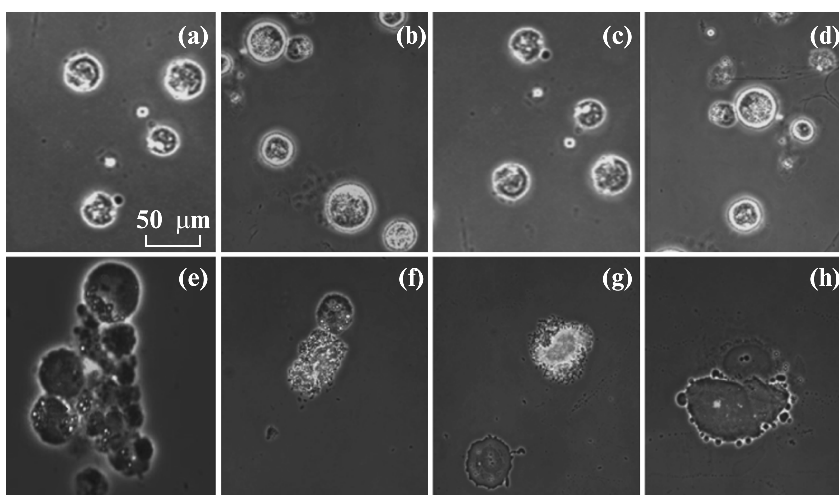

Fig. 2. Effect of magnetic nanodisks modified with the DNA aptamers in a rotating magnetic field on Ehrlich ascites carcinoma cells. Changes in the cell morphology were recorded 90 min after the exposure to the rotating magnetic field. (a) Ascites cells that were exposed to the magnetic field for 20 min; (b) ascites cells with the unmodified magnetic disks; (c) ascites cells with the DNA aptamers that were exposed to the magnetic field for 20 min; and (d) ascites cells with immobilized nanodisks modified with the DNA aptamers in a ratio of 1 : 5 (cells : nanodisks) that were not exposed to the magnetic field. In all control samples, destroyed cells were virtually absent. (e–h) Ehrlich ascites carcinoma cells with immobilized magnetic nanodisks modified with the DNA aptamers 90 min after the exposure to the rotating magnetic field. Cases of necrosis and apoptosis of cells in these samples can be seen.

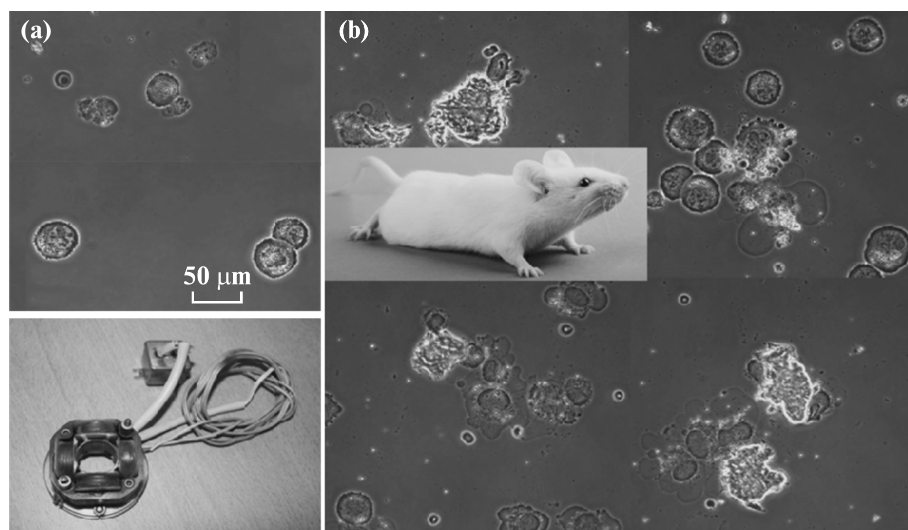

Fig. 3. Ehrlich ascites carcinoma cells isolated from the ascitic fluid of (a) control mice and (b) mouse injected with the magnetic nanodisks modified with the DNA aptamers 90 min after preliminary exposure to the rotating magnetic field. Cases of necrosis and apoptosis can be seen.

cells were washed twice with DPBS buffer, and their functional state was assayed with a FluoView FV10i confocal microscope.

The effect of the modified nanodisks in vitro and in vivo was similar: in the ascites cell samples taken from the tumor-bearing mice, the destruction of tumor cells was observed despite the fact that the ratio of disks and Ehrlich ascites carcinoma cells was approximately 1 : 10 (disks : cells, Fig. 3). This fact indicated a high efficiency of magnetic Ni-nanodisks modified with the DNA aptamers and the rotating magnetic fields for targeted cell surgery.

Thus, the simple technologies for obtaining nanodisks and their modification with the DNA aptamers open a new direction in the interdisciplinary research aimed at developing biologically functional hybrid nanodevices based on metals, magnetic materials, and DNA aptamers, which interact with molecular and

cellular targets in a targeted manner, and respective instruments and methods.

ACKNOWLEDGMENTS

This work was supported by the Russian Science Foundation (project no. 14-15-00805).

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Translated by M. Batrukova