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NANOBIOMEDICINE
AND NANOPHARMACEUTICALS

Magnetic Nanodiscs That Destroy Glioblastoma Cells in a Targeted Way in an Alternating Nonheating Magnetic Field

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Abstract—The need to develop a surgical instrument that can most effectively and minimally invasively remove a malignant tumor, and distinguish and destroy only tumor cells without damaging the normal cells of healthy tissue surrounding the tumor is being considered. To achieve this goal, it is proposed to use nanodiscs with special magnetic, electronic and optical properties. Nanodiscs modified with recognition ligands (aptamers) are able to bind to tumor cells and destroy them under the influence of a weak, nonheating alternating magnetic field. This allows for effective tumor destruction while minimizing the impact on surrounding healthy tissue.

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INTRODUCTION

Oncological diseases are one of the main causes of mortality among the working-age population [1]. The problem of mainstream therapies such as surgery and radiation therapy is that they are highly invasive since they damage healthy tissue surrounding a tumor. This is especially dangerous when treating glial brain tumors. Another problem is the impossibility of completely removing all tumor cells, since they may remain invisible and contribute to the formation of new tumors [2]. To remove a tumor as radically as possible with minimal damage to healthy tissue, a tool is needed that can find and remove only tumor cells without damaging healthy ones. The creation of such a tool is possible using nanotechnology and nanomaterials with unique properties: electronic, optical, and magnetic [3]. Magnetic discs, called nanoscalpels, may be a promising surgical tool for destroying tumor cells [4]. These discs have a recognition ligand that can bind to tumor cells and destroy them under the influence of an alternating magnetic field (**MF**). The nanoscale tool of tumor removal consists of two components. The first, a nanoscalpel, causes damage to the tumor cell, leading to its death [5]; the second, the recognition element, interacts with the tumor cell, ensuring contact of the nanoscalpel with it. The need to use magnetic discs to destroy tumor cells is explained by the fact that the effectiveness of using superparamagnetic nanoparticles for this purpose has reached its limit. The size of superparamagnetic particles limits the magnitude of their magnetic response, which is necessary for biomedical applications [6]. However, when sizes exceed the superparamagnetic limit, particles begin to accumulate. In contrast, the efficiency of nanoscalpels, on the contrary, increases with increasing magnetic moment of the particle. Specially manufactured [4, 6] magnetic microdiscs and nanodiscs, in particular three-layer Au/Ni/Au [4], have a high magnetization saturation and the absence of residual magnetization, which allows the remote

control of particles using MF, avoiding the problem of disc accumulation [7]. As a result, these discs become ideal magnetomechanical tools for destroying cancer cells. Aptamers ideally serve as targeted transport molecules for delivering nanoparticles and nanodiscs to a tumor, and can also ensure their fixation on the cell membrane.

Glial tumors of the human brain are characterized by malignancy, which leads to high mortality [1]. The large-scale invasion of tumor cells into surrounding tissues makes it impossible to completely remove the tumor, and traditional methods are ineffective for its treatment [1]. This work evaluates the effectiveness of three-layer magnetic nanodiscs with a quasi-dipole structure (Au/Ni/Au), modified with the Gli-233 aptamer [8] under the influence of an alternating MF, for the destruction of a glial tumor of the human brain transplanted into the brain of immunosuppressed mice.

The further research and use of magnetic nanodiscs with recognition ligands will provide new opportunities for the development of tools for the most selective destruction of malignant tumor cells, opening the way to minimally invasive methods of cancer resection and treatment.

EXPERIMENTAL

Preparation of magnetic nanodiscs and their functionalization using aptamers. The Au/Ni/Au discs were obtained by optical lithography and electron-beam sputtering onto single-crystal Si substrates with a diameter of 100 mm. For the photolithography stage, an FN-16U-4 negative resist was used, which was diluted with AZ EBR Solver to a thickness of ~200 nm before application. Next, standard operations for preparing the photoresist were carried out according to the technological map for this type of photopolymer:

(i) application by centrifugation at a centrifuge rotation speed of 3000 rpm;

(ii) drying the resulting layer at a temperature of 110°C;

(iii) exposure through a preliminarily prepared template, the negative photopolymer was subjected to an additional temperature treatment procedure;

(iv) development of formed structures.

As a result of the performed operations, a geometry of the photoresist was formed in the form of columns with a height of 220 nm and a diameter of ~1000 nm (area is 2500 mm^2). After the optical-lithography process, metal layers of Au and Ni were deposited using electron-beam sputtering on an EBS installation.

The functionalization of nanodiscs with thiolated Gli-233 DNA aptamers capable of in vivo binding [8] and oligonucleotides that are nonspecific for glial cells was carried out according to the procedure described in [6]. They were incubated with 1 μM of thiolated oligonucleotides reduced in TCEP buffer for 24 h at 6°C on a shaker to stabilize the discs and prevent their conjugation.

Studies on a cell model in a microfluidic chip. Microfluidic chips were made of polydimethylsiloxane (**PDMS**) using an SU-8 resist ("soft lithography") according to the method [9] at Alferov Saint Petersburg National Research Academic University, Russian Academy of Sciences. In general, the process consisted of three stages: making a photomask; manufacturing a master mold from a photoresist deposited onto a silicon wafer; production of microfluidic chips. Production of the photomask was carried out on a DWL 66FS laser-lithography installation (Heidelberg Instruments Mikrotechnik GmbH, Germany) with a characteristic resolution of ~1 μm.

To form microstructures on the surface of the silicon substrate, SU-8 2025 and SU-8 2075 (MicroChem Corp., USA) resists were used, which made it possible to obtain films with a thickness of 10–250 μm using a Delta 6 RC centrifuge (SUSS MicroTec, Germany). After applying the resist, it was removed from the edge of the sample mechanically and annealed at a temperature of 95°C for 7–60 min, depending on the thickness of the layer. To increase the adhesion strength of SU-8 to the silicon substrate, the latter was pretreated in a 50% hydrofluoric-acid solution for 40 s. To cast PDMS replicas of microfluidic chips using the manufactured master molds, a Sylgard 184 kit (Dow Corning, USA) was used, consisting of a base and a hardener mixed in a 10 : 1 ratio. After degassing and pouring the mixture into the mold, it was cured at a temperature of 65°C for 4 h. At the end of the curing process, PDMS replicas of microfluidic chips were separated from the mold, and holes were made in them using a punch. After this, the surface of the replica and the protective glass plate in the form of a cover glass were treated in a V15-G oxygen-plasma installation (PINK GmbH Thermosysteme) and their subsequent connection was performed.

A diagram of the microfluidic-chip channels with cell traps is shown in Fig. 1.

Magnetomechanical therapy in microfluidic chips. For large-scale experiments to search for the optimal conditions for magnetomechanical therapy, Ehrlich ascites carcinoma cells in microfluidic chips were used as model cells. For filling, 1 mL-syringes were used. The sample was introduced using an AL-1000HP syringe pump (WPI, England). For therapy, magnetic discs conjugated with an aptamer to As-42 Ehrlich ascites carcinoma cells were used. The chip was placed in a coil with a variable MF frequency of 50 Hz and an intensity of 100 mT for 10 min. The measurements were carried out using an LSM 780 NLO confocal microscope (Carl Zeiss, Germany); photographs of the cells were taken using an AxioCam MRc 5 camera (Carl Zeiss, Germany).

The general diagram of a microfluidic chip with cell traps is shown in Fig. 1. The chip has two input

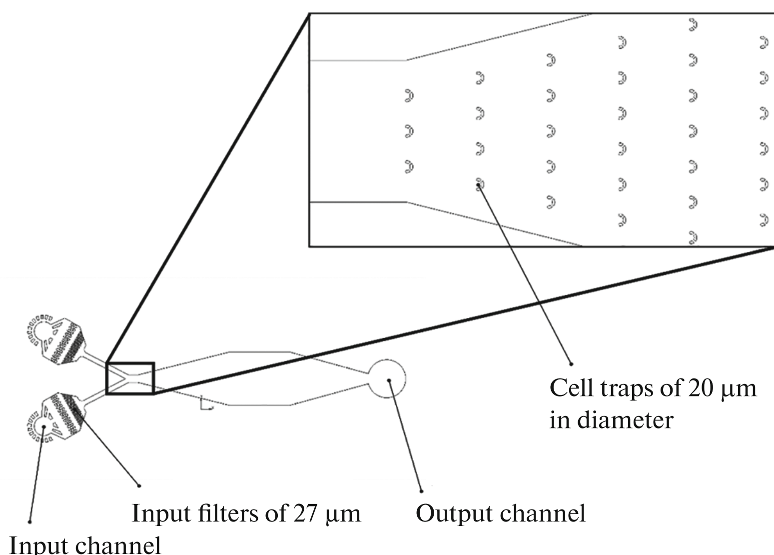

Fig. 1. Diagram of channels of a microfluidic chip with cell traps.

channels and one output channel. Each of the input channels is equipped with a filtration system. Cell traps with a diameter of 20 μm are located in the central chamber of the chip for cell fixation.

Development of a laboratory setup for controlling magnetic nanodiscs. A laboratory research facility for searching for the optimal modes of targeted destruction of target cells in low-frequency magnetic fields, carried out on experimental cell models of glial tumors, was developed specifically for this experiment (Fig. 2). The coil consists of Helmholtz rings with an average diameter of 6 cm and is connected to an AC generator with a control system. The coil can generate a MF with a maximum induction of 150 Oe. The installation allows one to change the shape of the alternating MF (rectangular or sinusoidal), frequency, voltage, and duty cycle of the rectangular alternating MF. A MF with a maximum induction of 200 Oe can be formed in the coils. The coils are connected to an AC generator. Each coil is connected to a separate generator channel. Separate generator channels allow the formation of both a homogeneous and inhomogeneous MF. The generator is made on an AtMega328P microcontroller and is connected to the computer via a USB controller with a virtual COM port function. (CP210x usb to uart bridge). The generator has a built-in 12-V 5-A power supply from a 220-V AC network and a fan for cooling. The creation of the appropriate shape and amplitude of the current is ensured by pulse-width modulation. The generator allows one to produce sinusoidal and rectangular signals. The following parameters are set for the signal: freq is a frequency from 0.1 to 1000 Hz, amp is an amplitude from 0.1 to 100, dFi is the phase difference between two coils in degrees, 0 means the phases are the same, and 180 means the signals are out of phase.

In vivo studies. To induce immunosuppression, cyclosporine at a concentration of 20 mg/kg and cyclophosphamide at a concentration of 60 mg/kg were intraperitoneally injected to animals five times with an interval of 48 h. Before administration, both drugs were diluted with saline to the required concentration, and every day the animals received ketocanazole orally at a dose of 20 mg/kg. After the fifth administration of the drugs, the orthotopic transplantation of glial cultures into mice was performed on the same day. The day after xenotransplantation, only cyclosporine was administered to the animals once. During the period of immunosuppression, in order to avoid fungal infections, an antifungal drug, Ketoconazole, was added to the drinking bowl at a final concentration of 25 mg/L.

The study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. The protocol was approved by the local committee on the ethics of animal experiments of Krasnoyarsk State Medical University No. 3 on December 16, 2022. All operations were performed under anesthesia, and all efforts were made to minimize animal suffering.

The subjects of the study were male laboratory mice weighing 20–25 g of the ICR line with immunosuppression. The formation of a human glioblastoma model was carried out by the intracranial injection of glioblastoma tumor cells obtained from a glioblastoma patient. Glial tumor growth was monitored using nuclear magnetic resonance (NMR) tomography. Antitumor therapy was carried out using Au/Ni/Au nanodiscs modified with the Gli-233 aptamer.

The selection of optimal MF parameters for microsurgery with magnetic nanodiscs was carried out on

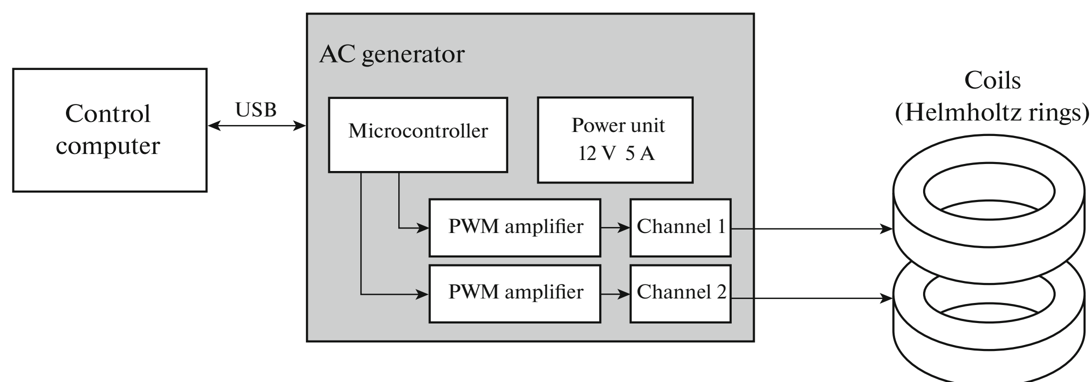

Fig. 2. Installation for controlling the magnetic nanodiscs.

Ehrlich ascites carcinoma cells in vitro. The effectiveness of magnetomechanical therapy was assessed on Ehrlich ascites carcinoma cell cultures in vitro at the following alternating MF frequencies: 1, 5, 10, 20, and 50 Hz (MF intensity is 100 Oe).

RESULTS

An experiment to assess the number of dead cells in cell traps was carried out after magnetomechanical therapy with magnetic nanoparticles and aptamers depending on the time after treatment. For this purpose, magnetic nanodiscs and the As-42 aptamer were used on Ehrlich ascites carcinoma cells as model ones. Data were recorded using an AxioCam HRc High Resolution digital camera (Carl Zeiss, Germany) on an LSM 780 confocal microscope (Carl Zeiss, Germany). A microfluidic chip with cell traps was filled with cells incubated with a nanodisc complex with As-42. The cell membrane potential dye $DiBAC_4$ (green) and the dead-cell dye Propidium iodide (red) were added to the PBS buffer medium, in which the cells were located. The chip with the cells was placed in the measuring chamber of a confocal microscope. A coil was installed on top of the chip, in which an alternating MF was generated with a frequency of 50 Hz and a strength of 10 mT. The membrane potential decreased in the alternating MF, and the cells died (Fig. 3), as evidenced by the nucleus stained with propidium iodide. Based on the performed experiments, the optimal characteristics of the alternating MF were selected: frequency of 10 Hz, strength of 150 Oe, and a sinusoidal field shape.

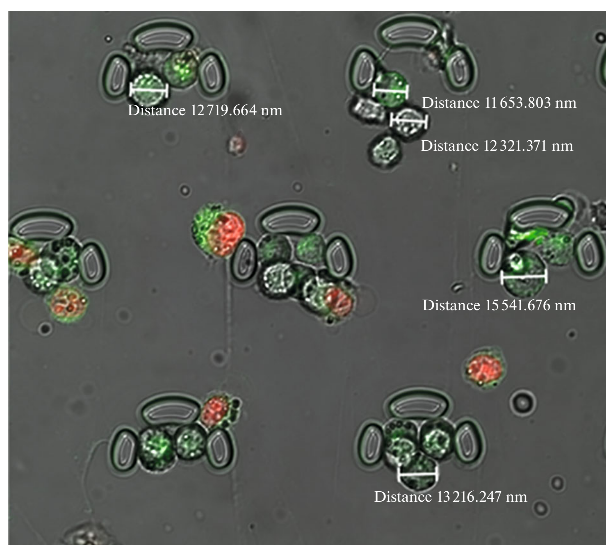

Fig. 3. Cells die 7 minutes after the end of exposure to a weak alternating magnetic field.

In vivo studies. To successfully carry out the orthotopic transplantation of glioblastoma, the animals were subjected to drug immunosuppression. A combination of the following drugs was chosen as immunosuppressants: cyclosporine, which selectively acts on T lymphocytes, and the cytostatic drug cyclophosphamide. As is known, in therapeutic concentrations, cytostatics can suppress the immune response. Assessment of the immune status of mice before xenotransplantation of glioblastoma into their brain showed that the immune regulatory index (CD3, CD4/CD3, CD8) of animals before tumor transplantation decreased significantly: from 7.4 ± 2.6 to 1.7 ± 0.6.

The development and treatment of glial tumors in mice were monitored using NMR tomography. The studies showed that within 14 days the tumor increased to a significant size (Fig. 4). The glial tumor after four sessions of magnetomechanical therapy using a “smart” nanoscalpel in an alternating MF (10 Hz, 150 Oe) gradually decreased (Fig. 4, A1–A3), while the glial tumors of mice treated with nanodiscs modified with nonspecific aptamers continued to increase in size (Fig. 4, B1–B3).

DISCUSSION

The most important biomedical task is the development of a “smart” nanoscalpel, consisting of a

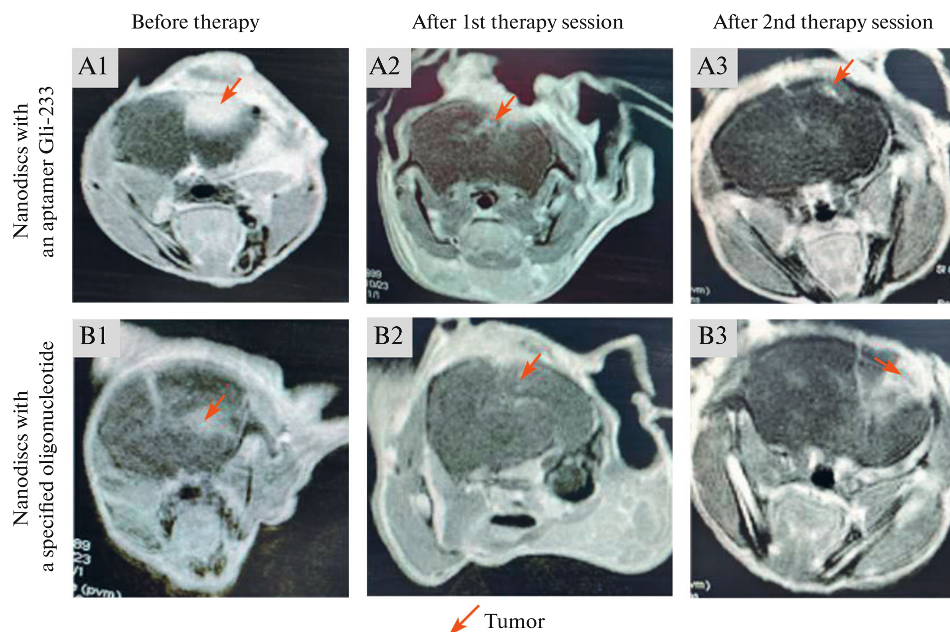

Fig. 4. Efficiency of a "smart" nanoscalpel in the microsurgery of glial tumors: tumors before treatment (A1, B1), treatment with nanodiscs modified with the Gli-233 aptamer (A2, A3), treatment with nanodiscs modified with a nonspecific aptamer (B2, B3).

nanosurgical instrument and tumor-recognizing molecules capable of selectively destroying only tumor cells in vivo. The function of nanosurgical instruments can be performed by nanodiscs with magnetic anisotropy and very high sensitivity to magnetic influences. Discs acquire greater magnetization in a weak external MF, since they have zero total magnetization without a MF. Targeting of the "nanoscalpel" to a target tumor cell is achieved by the functionalization of magnetic nanodiscs with DNA aptamers that are specific and have an affinity for tumor cells, which are used as bioidentifying molecules [10–12].

The use of microfluidic, cellular, and biochemical technologies and physical–technical solutions in combination makes it possible to automate and conduct mass experiments to search for optimal conditions for the magnetomechanical destruction of target cells, depending on the concentration and physical properties of magnetic nanodiscs, and the frequency, geometry, intensity, and time of action of the MF.

The penetration of magnetic discs into the cell occurs through endocytosis followed by encapsulation into lysosomes [13]. It is assumed that rupture of the lysosomal membrane under the influence of an alternating MF is the cause of cell death [14, 15], while the penetration of discs into cells without the influence of an alternating MF does not reduce the viability of tumor cells. The entry of discs into the cell begins with interaction with cell-membrane proteins, which are specific targets for aptamers.

Consequently, one of the most important tasks in developing a "smart" nanoscalpel for the microsurgery of malignant tumors is the problem of selecting the number of nanodiscs sufficient to destroy all tumor cells, since it is well known that tumor cells double almost every 24 h. However, there is the problem of eliminating the tumor too quickly. Destruction products can be toxic to the body and pose no less danger than living tumor cells. Despite the fact that the method of magnetomechanical therapy seems very promising, there are still limitations to its use associated with the removal of dead cells from the body. If magnetic microsurgery is used as an additional tool during surgery, destroyed cells and discs can be removed by simple washing. Thus, the obtained results showed the fundamental ability of nanodiscs to target and destroy individual tumor cells.

In the future, it is planned to improve this method for its use in the intraoperative removal of individual tumor cells to prevent tumor recurrence during the resection of solid tumors with the subsequent washing of discs and destroyed cells. The use of discs for this procedure is primarily due to the fact that the discs are not biocompatible and, due to their large size, are difficult to remove from the body. The use of magnetic discs intraoperatively is a promising technology for the treatment of malignant neoplasms, since the development of modern technologies makes it possible to obtain discs for tumor therapy on an industrial scale.

CONCLUSIONS

Evaluation of the effectiveness of an antitumor therapy using a "smart" nanoscalpel showed that microsurgery using three-layer magnetic nanodiscs

with a quasi-dipole structure (Au/Ni/Au) modified with a glial cell-specific aptamer Gli-233 (“smart” nanoscalpel) is effective for the treatment of glial tumors of the human brain.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the local committee on the ethics of animal experiments of Krasnoyarsk State Medical University No. 3 on December 16, 2022. All operations were performed under anesthesia, and every effort was made to minimize animal suffering.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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