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THE DEVELOPMENT OF NEUROSCAFFOLD FOR THE GLIOBLASTOMA THERAPY

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Abstract

Current paper presents the results of the system development for intracranial implantation aimed on therapy and prevention of brain gliomas relapse. The main property of the system, in prospective, will be to direct the growth of glioma cells localized in the region adjacent to the site of the removed tumor along the fibers towards the proximal part of the fiber-optic scaffold (neuroport). Such approach will allow carrying out cells diagnostics by the photoluminescence signal and provide subsequent destruction of malignant cells by photodynamic action. Besides, this system could be used for monitoring the processes occurring in the probed area in order to control the possible relapses. The localization of cells along the fiber structures covered with gelatin compound, which is the source of amino acids during cultivation, was shown during the glioma cells growth dynamics study. Moreover, four different designs of intracranial scaffold models, serving as ports for diagnostic and therapeutic laser radiation delivery, were developed and successfully tested in the framework of the research. The results obtained on the rats brain with induced tumors (glioma C6) after neuroport implantation demonstrate sufficiently intense fluorescence in the tumor bed after intravenous injection of the nonmetallic sulfonated phthalocyanine based photosensitizer, and a pronounced photodynamic effect leading to total destruction of the tumor. In this way, the results of this study open the prospects of creating the neuroport with an internal fiber structure that focuses the glioma cells growth.

Key words: spectroscopy, fluorescent diagnostics, photodynamic therapy, fiber-optic scaffold, nonmetallic sulfonated phthalocyanine, neurophotonics, glioblastoma.

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РАЗРАБОТКА И ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ НЕЙРОПОРТА ДЛЯ ТЕРАПИИ ГЛИОМЫ ГОЛОВНОГО МОЗГА

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Резюме

В работе представлены результаты разработки системы для внутричерепной имплантации с целью терапии и предотвращения рецидивирования глиом головного. Основное свойство системы в перспективе будет состоять в том, чтобы направить рост клеток

глиомы, локализованных в области, прилегающей к месту удаленной опухоли, вдоль волокон по направлению к проксимальной части волоконно-оптического имплантата (нейропорт) с целью их регистрации по сигналу фотолюминесценции и последующей их деструкции в результате фотодинамического воздействия. Такое устройство должно обеспечить мониторинг процессов, происходящих в зондируемой области с целью контроля процессов рецидивирования. В ходе данного исследования динамики роста клеток глиомы показана локализация клеток вдоль волоконных структур, покрытых желатином, который является источником аминокислот при культивировании. Также в ходе работы были разработаны и успешно апробированы четыре различных конструкции макетов внутричерепных имплантатов, выполняющие роль портов для доставки диагностического и терапевтического лазерного излучения. Получены на головном мозге крыс с индуцированными опухолями (глиома С6) после имплантации нейропорта, демонстрирующие достаточно интенсивную флуоресценцию в ложе опухоли при внутривенном введении фотосенсибилизатора на основе безметального сульфированного фталоцианина и выраженный фотодинамический эффект, приведший к тотальному разрушению опухоли. Полученные результаты открывают перспективы создания нейропорта с внутренней волоконной структурой, фокусирующей рост клеток глиомы.

Ключевые слова: спектроскопия, флуоресцентная диагностика, фотодинамическая терапия, безметальный сульфированный фталоцианин, оптоволоконный имплантат, нейрофотоника, злокачественная глиома.

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Introduction

Currently, the general treatment of patients with glial brain tumors is characterized by a comprehensive approach that includes surgical removal, radio- and chemotherapy [1, 2]. Other methods, such as immunocorrective therapy and specific antitumor immunotherapy, being developed in some clinics, are not considered the standard and are still at clinical trials stage [3-5]. Despite the improvement of the surgical intervention methods and the enhancement of technical equipment capacity of clinical units performing postoperative radio- and chemotherapy, the results of combined malignant gliomas treatment have not significantly improved. The median overall survival of patients with multiform glioblastoma is 14 months. A number of factors determines the high mortality rate, one of which is the deep invasiveness of the multiform glioblastoma. Due to the specific infiltrative growth of such neoplasms type, the key shortcoming of surgical intervention is the lack of total tumor removal feasibility. That circumstance is the major reason leading to disease recurrence. It is significant to note that at present there are scaffolds that are placed into the tumor bed after resection. This therapy approach is based on the prolonged action of chemotherapeutic drug, which is gradually released from the scaffold, affecting the tumor cells remaining after surgical intervention thereby preventing recurrence [6-7]. Such way of brain neoplasms therapy is considered the most effective among the currently existing ones, however, some glioma types are resistant to chemo- and radiotherapy, which does not allow this method to be universal. Malignant brain gliomas are known to invade and spread along the white matter channels and blood vessels [8-10]. Due to the characteristic features of infiltrative growth along nerve fibers and vessels, the definition of primary intracerebral tumors boundaries is a particularly difficult task [11-12]. The recent studies of American scientific groups have shown that C6 glioma cells migrate massively along the polymer nanofibers coated with nutrient media. Furthermore, scientists achieved significant reduction of the tumor intracranial volume by implanting the fiber structures inside the cranium and the externally affecting with the chemo based on cyclopamine gel [8]. Based on this scientific observation, the creation of conditions for centripetal residual tumor cells proliferation tendency, not centrifugal (from the center to the periphery), appears to be perspective. Optical fibers, structurally imitating the white matter channels and blood vessels, are particularly relevant for such a system that sets the direction of tumor growth. Photodynamic therapy is the most appropriate and effective method for providing a destructive impact on pathological tissues due to the fiber-optic internal structure of the scaffold.

In this way, the major goal of this study is to create a system the main property of which is to provide the direct growth of glioma cells, localized in the region adjacent to the site of the removed tumor, along the fibers towards the proximal part of the fiber-optic scaffold (neuroport). Such configuration will allow malignant cells registration by the photoluminescence signal and their subsequent destruction as a result of photodynamic effect [10]. Thus, the neuroscaffold should be based on a biocompatible material framework with an internal fiber structure that focuses the glioma cells growth by branching from the proximal end to the distal one. Such a device will also provide monitoring of processes occurring in the probed area in order to control the relapse processes.

Materials and methods

Biological material

To conduct the study of cells localization under incubation conditions with optical fibers structures *in vitro*, a culture of rat brain tumor cells C6 was used. Cells were maintained by subculture method in RPMI medium.

Then, the cells were taken off the plastic culture flasks surface using trypsin. Tumor cells were incubated with optical fibers in the flat-bottomed culture panels during 5-7 days at 37°C until a monolayer was obtained.

For biointegration study of the developed scaffold framework, a series of experiments was conducted on mature Wistar rats models with 200-220 g mass at the beginning of the experiment, in which multiform Glioblastoma was modeled by stereotactic implantation of 5x105 cells of C6 glioma line into the striatum region [13]. Dynamic magnetic resonance imaging (MRI) of rats' brains was carried out weekly, starting from the 5th day after glioblastoma modeling, by tomograph ("BioSpec 70/30" Bruker, Germany) with 7 T permanent magnetic field. Ketamine anesthesia was injected into the femoral vein of rats at a dose of 100 mg/kg. The photosensitizer was then injected into the tail vein in a dose of 5 mg/kg of the experimental animal under the anesthesia.

The investigated photosensitizer

Phtalosens (Scientific Research Institute of Organic Intermediates and Dyes "NIOPIK") was used as a photosensitizer based on nonmetallic compound of sulfonated phthalocyanine, which is considered to be a good photocatalyst of oxidation processes, which is essential for effective fluorescence diagnostics and photodynamic therapy. The photosensitizer was injected into the experimental animals intravenously at 5 mg/kg dose.

Methods

The growth dynamics research of C6 glioma cells under incubation conditions with the optical fibers structures *in vitro* was carried out using confocal microscopy (LSM-710-NLO Carl Zeiss, Germany).

Experimental studies *in vivo*, devoted to monitoring and correction of conditions of laboratory animals with C6-induced glioma after intracranial implantation of the developed scaffolds of various design, were performed by fluorescence diagnostics method with the use of LESA-01-BIOSPEC spectrometer (Russia), which has proved its efficiency in clinical trials [14-15]. Moreover, tumor growth and intracranial scaffold behavior in contact with the tumor tissue were also visualized and monitored by Magnetic resonance imaging (MRI) method using "BioSpec 70/30" tomograph (Bruker, Germany). Fluorescent diagnostics with the nonmetallic sulfonated phthalocyanine derivative photosensitizer (c=5mgkg) was carried out by laser radiation with 100 mW/cm^2 power density and λ=632.8 nm. Photodynamic therapy with the use of a nonmetallic sulfonated phthalocyanine derivative (c=5 mg/kg) was also carried out by λ=675 nm laser radiation in three 10-minute approaches with two-minute intervals for fluorescent diagnostics with a total dose of 200 J/cm$^{2.}$

Results and discussion

Results of in vitro studies

The growth processes of C6 malignant glioma cells and their localization on the optical fibers surface, which in the plans will constitute the internal structure of the brain scaffold, were visualized by laser scanning confocal microscopy method. *In vitro* studies showed that malignant glioma cells form agglomerates around the optical fibers, create bindings and localize directly along the fiber structures (Fig. 1). The obtained results

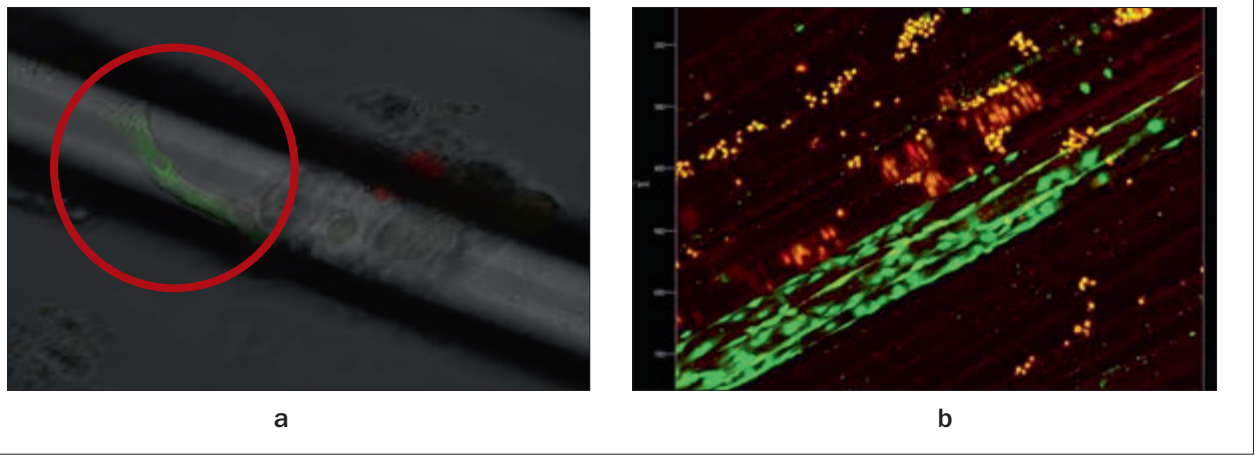

Fig. 1. 3D-reconstruction of fluorescent images showing the C6 glioma cell growth along the optical fiber:
a – single C6 glioma cell (highlighted area);
b – C6 glioma cell clusters, stained with acridine orange (AO) (Green – living cells) and propidium iodide (PI) (Red – dead cells)

Рис. 1. 3D-реконструкция флуоресцентных изображений роста вдоль оптического волокна:
a – единичная клетка глиомы C6 (выделенная область);
b – скопления клеток глиомы C6, окрашенных акридиновым оранжевым (AO) (зеленый цвет – изображение живых клеток) и пропидий-йодидом (PI) (красный цвет – изображение погибших клеток)

suggest that the approach of directed malignant glioma cells growth from the intracranial region into the depth of the developed scaffold with subsequent photodynamic impact leading to cell death can be realized *in vivo* conditions.

During the course of the study, the scaffold design was optimized to provide the most favorable conditions for carrying out fluorescence diagnostics and photodynamic therapy (Fig. 2, 3). Moreover, the biointegration process of scaffolds made of various promising biocompatible materials (hydroxyapatite, sapphire and polymeric materials) was investigated (Fig. 4).

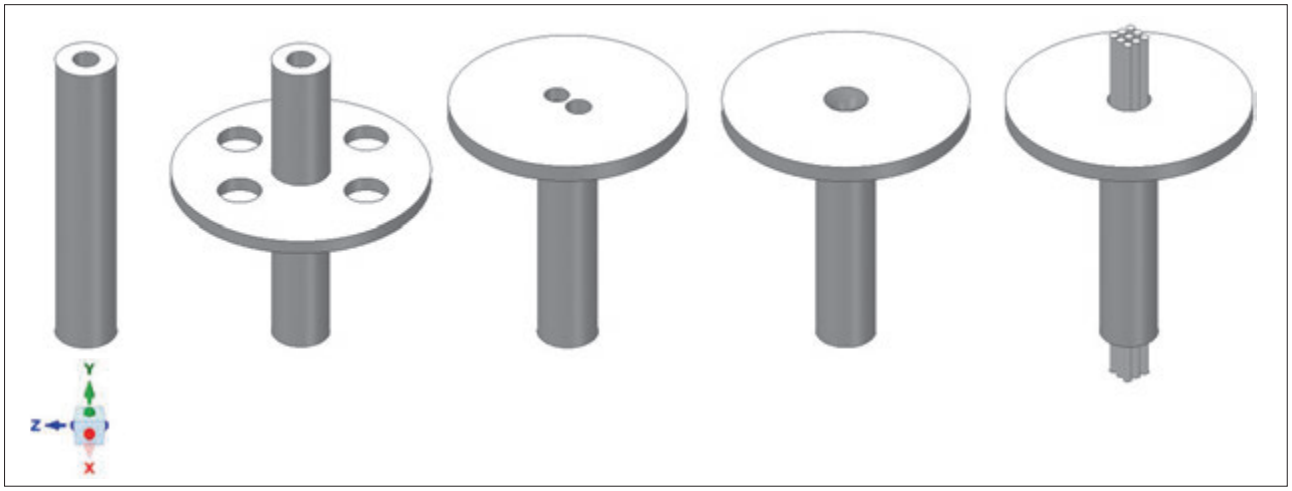

Fig. 2. Scheme of neuroscaffold design evolution
Рис. 2. Схематическое изображение эволюции конструкции нейропорта

Results of in vivo studies

During the study, different designs of scaffolds made of various materials were developed and tested on experimental animals. Scaffolds of various designs, hollowed and with internal fiber structure, were predesigned and adapted to the scale of the fiber-optic probe (Fig. 2). Samples were installed inside the cranium of the experimental animals and served as ports for local delivery of diagnostic and therapeutic laser radiation (Fig. 3).

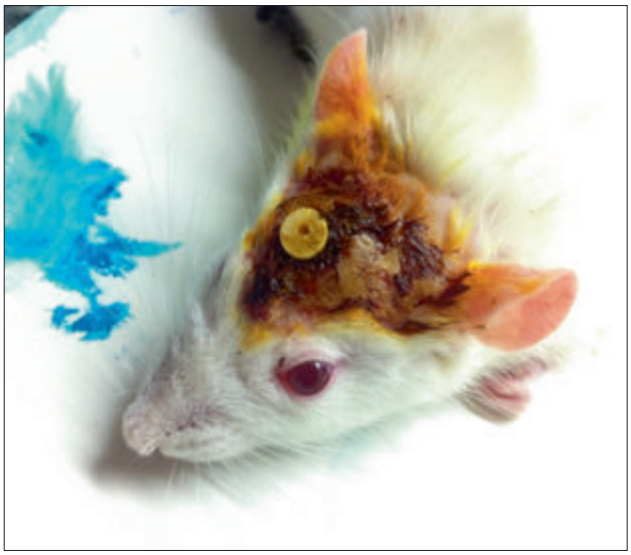

Fig. 3. Intracranial neuroscaffold device mount.
Рис. 3. Крепление нейропорта на черепной коробке экспериментального животного

The process of scaffold biointegration was monitored by MRI method. The optimal properties and nature of material for neuroport manufacturing were identified by a series of experiments. According to MRI results, the absence of extensive abscesses and scaffold rejection was established only in case of polymer structures implantation (Fig. 4). However, this obstacle does not mean that hydroxyapatite- and sapphire- based scaffolds are not biocompatible, though the quality requirements and the cleaning degree of their surface should be higher. Successful approbation allowed determining the optimal properties and external size of the brain scaffolds for experimental animals: polymer PLA d=3 mm, h=5 mm. It is worth noting that designs based on biocompatible PLA material were produced using 3D printing that makes it easy to vary the scaffolds sizes.

Polymeric constructions with an internal fiber-optic structure of the scaffolds were fixed subcutaneously on the skull so that the scaffold frame and the end of the fiber-optic interior part were set into the tumor bed of the experimental animals (Fig. 4c). The scaffolds placed into the tumor bed acted as a port for the local diagnostic and therapeutic laser radiation delivery that allowed monitoring of the experimental animals condition after implantation. Control was carried out by fluorescent diagnostics based on the nonmetallic sulfated phthalocyanine photosensitizer at λ_{ex}=632.8 nm excitation (Fig. 5, 6), and by MRI (Fig. 7). It was shown that the internal

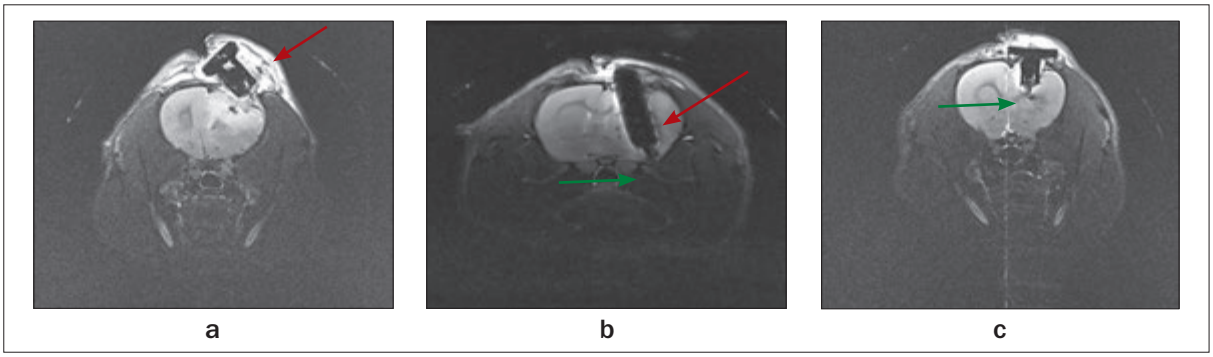

Fig. 4. MRI T2 mode scans of the rat's brain on the 5th day after neuroscaffold implantation; the devices are made of:
a – hydroxyapatite;
b – sapphire;
c – polymer PLA
Red arrows mark areas of inflammation; green arrows mark areas of protruding fiber part
Рис. 4. МРТ-снимки головного мозга крысы в режиме T2 на 5-е сутки после имплантации конструкций с внутренней оптоволоконной частью и внешней частью, изготовленных из различных перспективных биосовместимых материалов:
a – гидроксиапатит;
b – сапфир;
c – полимер PLA
Красными стрелками отмечены проблемные области в процессе биоинтеграции нейропорта. Зелеными стрелками выступающая часть внутренней волоконной структуры нейропорта

fiber structure allows tracing the cells growth into the deep of neuroport by spectral methods. However, the experiment complexity of MRI evaluation of C6 glioma cells migration into the depth of neuroport is associated with the need for the long-term monitoring that leads to extensive accompanying tumor growth outside the scaffold. Nevertheless, the high sensitivity and efficiency of spectral methods for the early glioma cells detection in the immediate vicinity of the scaffold by fluorescent signal has been experimentally established. Hollowed polymeric structures were also tested in experimental animals. Hollowed scaffolds were fixed subcutaneously

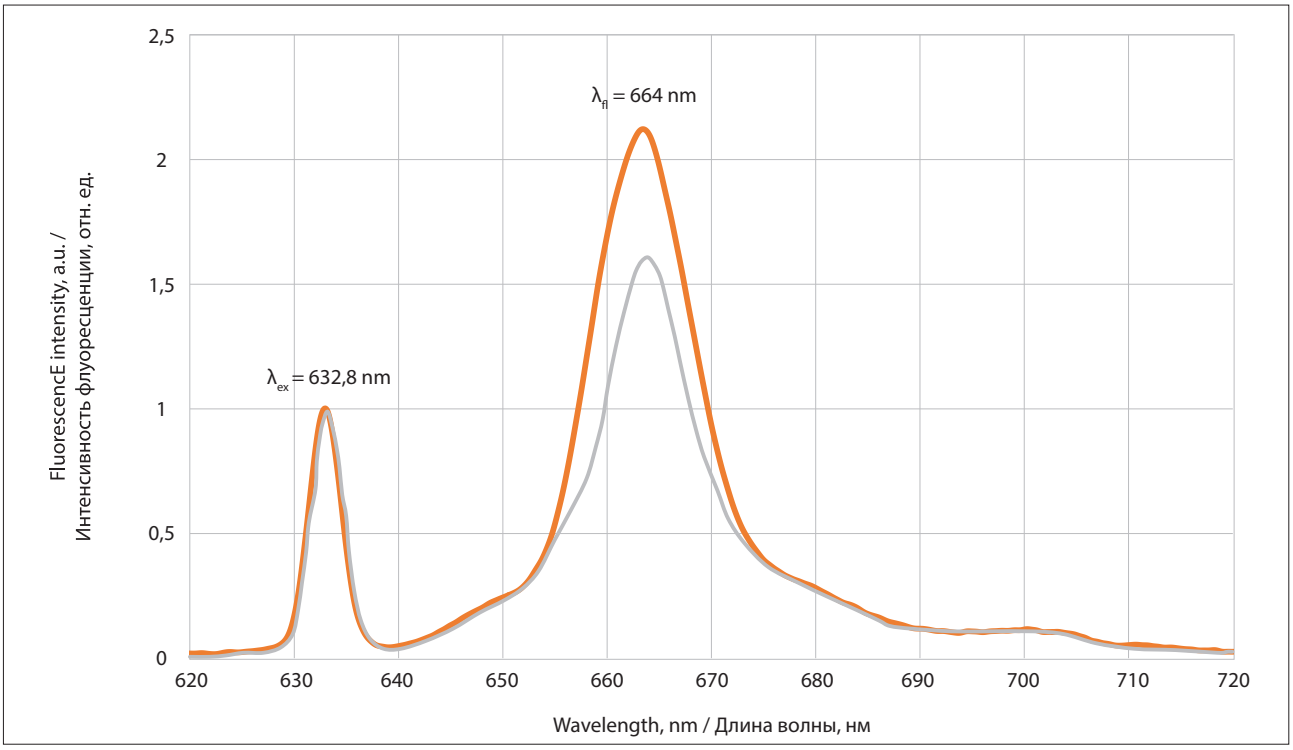

Fig. 5. The fluorescence spectra, characterized by the drug (nonmetallic sulfonated phthalocyanine) accumulation in the tumor (λ_{ex} = 632.8 nm) obtained for –hollowed and –fiber-optic neuroscaffolds
Рис. 5. Спектры флуоресценции, снятые с помощью имплантатов с –полой и –внутренней оптоволоконной структурой, характеризующие уровень накопления фотосенсибилизатора на основе безметального сульфированного соединения фталоцианина в опухоли (λ_{ex}=632,8 нм)

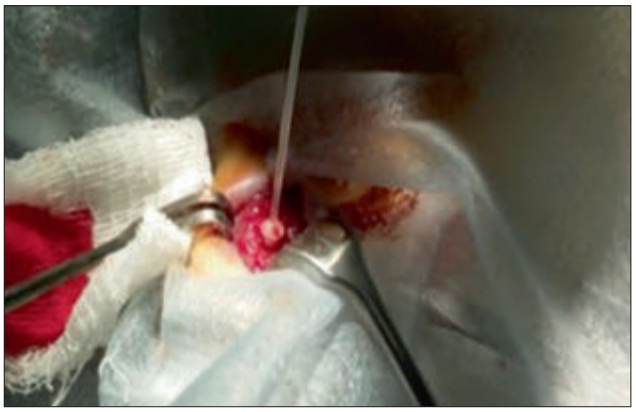

Fig. 6. Fluorescence diagnostic mode using phthalocyanine series (λ_{ex}=632.8 nm)
Рис. 6. Проведение флуоресцентной диагностики с безметальным сульфированным фталоцианином (λ_{ex}=632,8 нм)

on the skull so that the scaffold frame and the cavity were located into the rats' tumors bed (monitoring was carried out by MRI (Fig. 7a)). The high therapeutic effect was obtained because of photodynamic therapy (with nonmetallic sulfonated phthalocyanine, excitation by radiation with a wavelength of 675 nm) and was assessed by MRI. In Fig. 7a the MRI image shows the presence of tumor in the striatum region, which is a light section with the corresponding localization indicated by an arrow. In Fig. 7b the MRI image demonstrates the therapeutic effect of photodynamic therapy in the implantation area which is uniform staining of the tissues in the striatum region indicated by an arrow. The result of photodynamic therapy was assessed by MRI for 2 weeks with a 3 day period. MRI photograph shown in Fig. 7 demonstrates the photodynamic therapy efficiency obtained on the 6th day after PDT. As a result of photodynamic therapy a high therapeutic effect (survival score in comparison with the control group) was achieved, and the constant access to the tumor bed was provided for condition monitoring (Fig. 7b).

Survival score was 8-9 weeks after glioblastoma modeling compared to 5-6 weeks of the control group.

Conclusion

It was shown under *in vitro* conditions that the C6 glioma cells are localized on the optical fibers surface during the growth process which gives the reason to expect that the approach of directed malignant glioma cells growth from the intracranial region into the developed within the project scaffold interior can be realized *in vivo* and followed by photodynamic impact, leading to cells death.

Various designs of neuroports with an internal fiber-optic structure were developed and integrated into the diagnostic monitoring of the rats' brain tumor processes *in vivo*.

MRI studies confirmed that the best therapeutic effect was achieved in the case of photodynamic therapy session with nonmetalic derivative of sulfonated phthalocyanine (c=5 mg/kg) using a polymer scaffold by λ=675 nm laser radiation delivering and 200 J/cm^2 dose.

The developed design of the scaffold with photosensitizer-coated optical fibers made it possible to implement a new approach to the brain gliomas diagnostics and therapy, including cases with high degree of malignancy.

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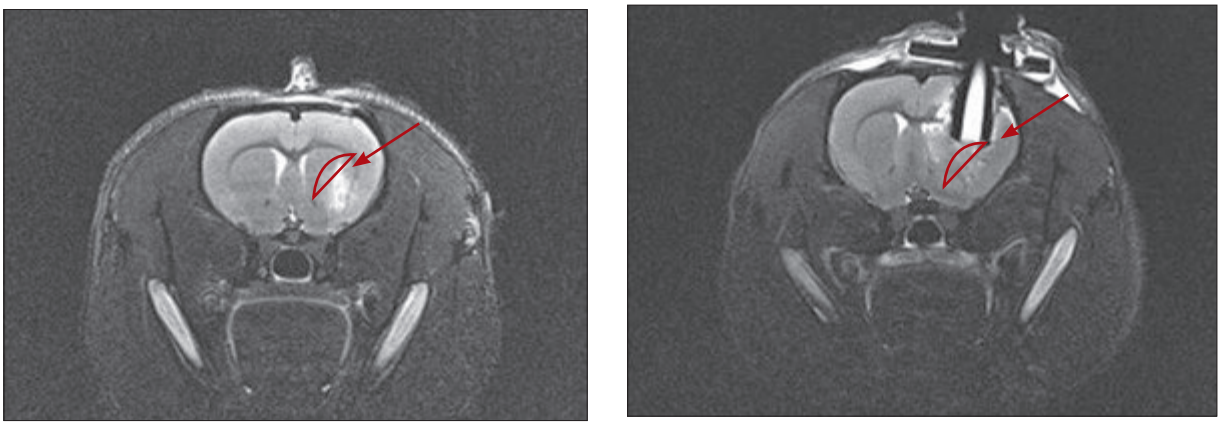

Fig. 7. MRI T2 mode scans of the rat's brain:
a – before implantation and treatment;
b – after implantation and photodynamic therapy by the nonmetallic sulfonated phthalocyanine (λ=675 nm)
Areas of C6 glioma location are marked
Рис. 7. МРТ-снимки головного мозга крысы в режиме Т2:
a – до имплантации и терапии;
b – после имплантации и ФДТ с безметальным сульфированным фталоцианином (λ=675 нм)
Стрелкой показано место локализации опухоли

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