Sensors

An integrated sensor system based on the phenomenon of surface plasmon resonance for early diagnosis of postoperative progression of malignant brain tumors

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Abstract. The integrated sensor system based on the phenomenon of surface plasmon resonance (SPR) is proposed. The system consists of a "Plasmon-6" biosensor device (model 321), a plunger pump, and an air dryer, placed in a thermal box. Stabilization of the temperature of the biosensor sensitive element is essential for studying biological media. The sensor system was used to confirm the completeness of surgical removal of a malignant brain tumor of grade II by monitoring the changes in the aggregation of peripheral blood cells mixed with a solution of verapamil hydrochloride. For this, the SPR indices of blood cells taken before and on the 7th day after the surgery were compared in 12 patients. A decrease in the SPR index after the surgery indicates complete resection of the malignant brain glioma, and an increase in the SPR index suggests the presence of micro-inflammation associated with tumor remnants. The latter means incomplete resection of glioma and indicates a high probability of recurrence due to tumor progression. The successful removal of brain glioma, as noted in the operation protocol, was confirmed for 7 patients, whereas for 5 patients the resection was incomplete. The advantages of using the proposed sensor system to assess the completeness of malignant tumor resection are quickly obtaining SPR indices, sensitivity to small doses of substances, safety for patients, autonomy and mobility. This allows us to take timely measures to prevent the postoperative progression of a malignant tumor.

Keywords: integrated sensor system, surface plasmon resonance, brain glioma, completeness of tumor resection.

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1. Introduction

Recently, in the fields of biology and medicine, new diagnostic methods and tools based on the optical phenomenon of surface plasmon resonance (SPR) in metal films have been developed [1, 2]. Among various physical phenomena related to the polarization properties of electromagnetic waves, this method is gaining increased scientific and practical importance. The term SPR refers to the resonant excitation of plasma waves of free electrons in the near-surface layer of a metal. The SPR phenomenon, used in these devices and diagnostic systems, allows for recording the surface interactions between biological molecules without the need for radioactive or enzyme labels [3, 4]. This makes it useful for diagnosing and assessing the progression of oncological diseases.

Particularly, the confirmation of the completeness of resection of brain glioma is an urgent task for modern neurosurgery [5]. Unfortunately, it is impossible to guarantee complete resection of tumor tissue in the brain due to its proximity to vital brain structures. At the same time, the main task of surgical intervention is to ensure the complete resection of the tumor and further monitor it to prolong and improve the quality of patients' life. A false conclusion regarding the absence of tumor remnants may lead to further tumor growth due to a lack of timeline treatment. Therefore, the development of a modern sensory system capable of assessing the completeness of surgical tumor resection is a critical issue in the fight against the most dangerous cancers.

Currently, a range of radiological diagnostic methods is used to detect tumor cells in brain tissue, including conventional X-ray imaging, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) [6]. The success of brain tumor treatment is largely determined by the level of completeness of surgical resection. To evaluate the completeness of resection, various tools are employed, namely transcranial endoscopic access for treating tumors in the third ventricle, along with methods to assess the spread of malignant glioma proliferation, including optical visualization systems. The completeness of brain tumor resection, which determines the possibility of its subsequent growth, can be assessed using MRI with contrast (using a gadolinium-based contrast agent). Additionally, perfusion MRI and positron emission tomography (PET) with amino acids are used to identify 'hot' nodules for tumor biopsy, especially when an open biopsy with tumor resection is not performed.

It is well known that inflammation is closely associated with all stages of development and the malignant progression of most types of cancer, as well as with the effectiveness of anticancer therapy [7]. Specifically, tumor-associated inflammation plays a crucial role in the mechanisms of brain glioma progression [8]. At the same time, in the case of incomplete glioma resection, micro-inflammation develops at the site of tumor remnants and causes an increase in the degree of peripheral blood cell aggregation.

The phenomenon of blood cell aggregation consists in the unification of blood cellular elements into one system due to the ability of erythrocytes to form "coin columns" and conglomerates of various sizes and densities in the circulatory system. A change in the level of blood cell aggregation causes a change in the effective dielectric constant of the erythrocyte mass solution. When blood cells are placed on the surface of a metal film of the SPR sensor, the changes in the aggregation level of these cells lead to changes in plasmon characteristics, namely frequency, line width, and shape of resonance curve. Analysis of changes in plasmon characteristics can be used to assess the completeness of glioma resection based on blood cell aggregation level.

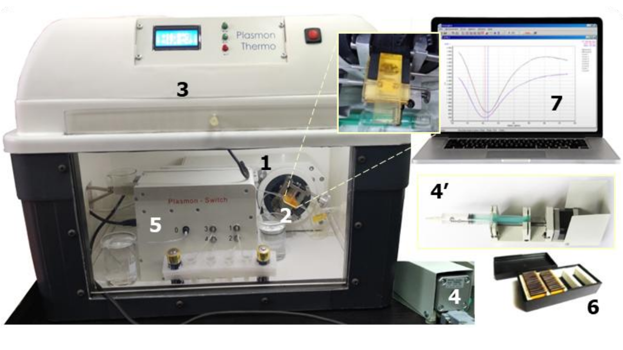

Fig. 1. Appearance of the integrated sensor system based on the SPR sensor. A description of the system components is provided in the text.

Our recent studies have confirmed the effectiveness of using a dual-channel SPR sensor ("Plasmon-6") to evaluate blood cell aggregation levels [9, 10]. However, it was also found that the surrounding environment significantly affects plasmon characteristics, emphasizing the need for temperature stabilization of the SPR sensor [9].

This work aims to develop a thermally stabilized integrated sensor system that allows determining the completeness of brain tumor resection by detecting changes in the levels of peripheral blood cell aggregation.

2. Set of the sensor system

Fig. 1 shows the developed integrated sensor system based on the SPR phenomenon for the early diagnosis of postoperative brain tumor progression. The integrated sensor system consists of the "Plasmon-6" (model 321) biosensor device (*1*), a dual-channel flow cuvette with a sensitive element based on an SPR slide (*2*), a thermal box (*3*), the pumps for circulating analytes through the flow cuvette of the device (*4*, *4′*), a channel switch for automating the delivery of analytes to the cuvette, an air dryer (*5*), a set of sensitive SPR slides for measurements (*6*), and a software (*7*). The range of resonance angles measured by the "Plasmon-6" (model 321) is within 38…69 ang. deg., corresponding to the change in refractive index within 1.00…1.41. The angular resolution of the biosensor device is 3 ang. sec., corresponding to the error in determining the refractive index at 1×10^{-5}. The sensitivity of the SPR sensor is within 95…100 ang.deg./RIU (refractive index unit). The advantages of SPR sensors are sensitivity to small doses of substances (≥1 nM) and an improved signal/noise ratio equal to 2500.

The sensitive element of the biosensor is a SPR slide made of F1-grade glass (refractive index n_D = 1.6128) coated with a gold layer of 30-50 nm thickness. The "Plasmon-6" biosensor device (model 321) has two optical channels that allow performing two different experiments in a differential mode using one channel as a reference. Differential measurements significantly enhance the device sensitivity by eliminating the influence of external factors (temperature changes, instability of laser emitter, *etc.*) on the measurement process. Additionally, placing the device in a thermal box helps stabilize the temperature of the biosensor sensitive element relative to the ambient temperature T within the range from – 5 °C to + 10 °C. The temperature stabilization minimizes the signal temperature drift and reduces its fluctuations caused by variations in the ambient temperature. This is especially important for studying biological media.

A plunger pump was designed to deliver the test solutions and the analytes to the sensory cuvette (Fig. 1, (*2*)). The plunger pump (Fig. 1, (*4′*)) provides easy and standardized contact between the analyte and the sensitive SPR layer of the sensory cuvette. The pump flow rate ranges from 1 to 5000 µL/min, which satisfies the requirements of many biological and medical studies.

The pump uses a standard 10 ml syringe, and the delivery of the analytes is controlled by a stepper motor. The pump design has several advantages: no metal-to-metal contact, no destructive effects from the environment on the pump, no impact of the pump on the analyte, minimal downtime and service maintenance, and easier installation and cleaning. Specifically, the only part subject to wear is the tubing, which can be replaced in less than a minute. The pump provides a high level of dosing accuracy and consistency, ensuring high measurement accuracy.

Various materials for the pump tubing (Teflon, polyethylene, silicone, *etc.*) were tested, and silicone was selected as the optimal material for research, as it offers the best wear resistance, biological and chemical inertness, and pump performance, as well as the ability of the visualization of analyte flow.

The pump is controlled using a specially developed computer program through the "Plasmon-6" biosensor device or using a dedicated standalone module. One or two pumps can be connected to the "Plasmon-6" device and programmed to perform a specific scenario of the experiment. Fig. 2 shows two examples of the software windows for monitoring pump operation. The system software allows the researcher to automatically adjust its parameters according to the requirements of the experiment, namely the speed and duration of analyte pumping, and so on.

The air dryer is integrated into the sensory system to remove moisture from the analyte delivery tubing and the measuring cuvette during the analysis.

The developed integrated sensor system allows standardizing experimental research of liquid analytes, including those for medical purposes. Specifically, the system was used for the investigation of completeness of tumor tissue resection. The results of these studies are presented below.

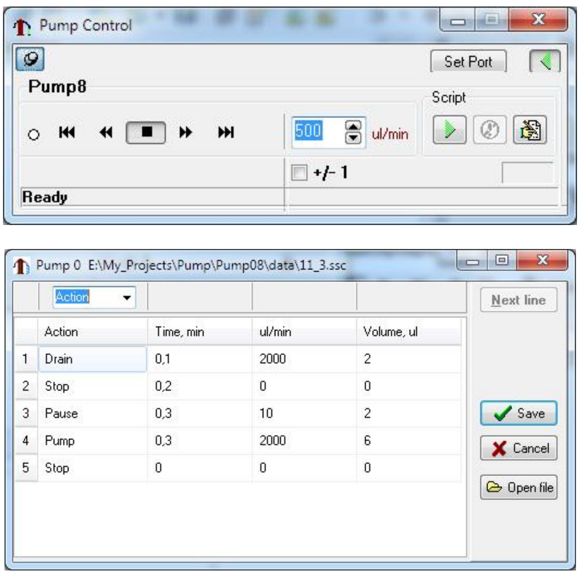

Fig. 2. Two examples of software windows for monitoring pump operation.

3. Study of the completeness of brain tumor resection: experimental results and their discussion

To study the completeness of tumor tissue resection, the SPR indices were measured before and after the surgery. In the last case, the blood taken on the 7th day after tumor removal was used. At this time, the inflammation

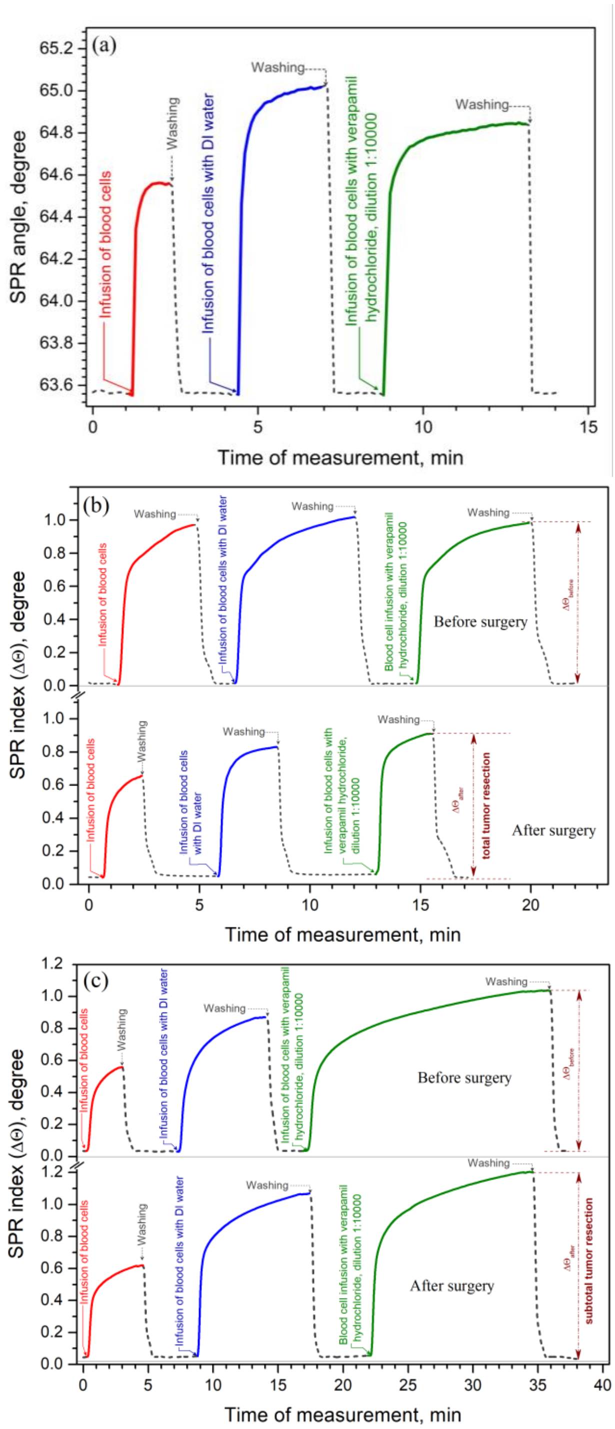

Fig. 3. Typical kinetic characteristics of the SPR angle variation during the blood cells analysis for healthy individual (a). The variation of the SPR index for the patients with grade II brain gliomas (b, c) measured before and after surgery that show complete (b) and incomplete (c) tumor resection.

caused by the surgery is the most pronounced, although the degree of micro-inflammation caused by incomplete tumor resection remains unchanged since the operation.

Fig. 3a shows the typical kinetic curves of the SPR angle changes in blood cells from healthy individuals. Fig. 3b and 3c show the variation of the SPR index for blood cells of the patients with brain gliomas taken before and after tumor resection.

The SPR index is defined as the change in the SPR angle caused by placing the analyte on the SPR slide compared to the SPR angle for deionized water. The analytes are the blood cells, the blood cells with added water (at a 10:1 ratio), and the blood cells mixed with a 0.25% solution of verapamil hydrochloride diluted with deionized water in a 1:10,000 ratio (at a 10:1 ratio). Blood cells were obtained by centrifuging heparinized blood (1 ml of heparin per 10 ml of blood) at 1500 rpm for 10 min. Since verapamil hydrochloride was dissolved in deionized water, a small amount of deionized water was also added to the blood cells for control. The addition of water to the erythrocyte mass at a 1:10 ratio does not cause damage to the erythrocyte membranes.

The earlier studies of malignant gliomas using SPR-based devices have shown that the higher the malignancy grade of the tumor, the greater the blood cell aggregation level, and the lower the SPR index [8, 11, 12]. At the same time, our recent research has shown that adding verapamil hydrochloride, diluted at 1:10,000, to the blood cells affects their aggregation level and the SPR index, and this effect is opposite for the patients with benign and malignant tumors [10, 13]. Verapamil hydrochloride is a calcium channel blocker that can alter the transmembrane potential across blood cell membranes, thus affecting blood cell aggregation [14, 15]. In the case of blood cells from a healthy individual or a patient with a benign tumor, adding verapamil hydrochloride increases blood cell aggregation. As a result, the SPR index of blood cells with verapamil becomes smaller compared to blood cells with water only (Fig. 3a).

In the case of blood cells taken from a patient with a malignant tumor, adding verapamil hydrochloride reduces the blood cell aggregation, and the SPR index of blood cells with verapamil becomes larger compared to blood cells with water (Fig. 3b). Thus, a larger value of SPR index of blood cells with verapamil compared to the blood cells without it can be used as a signature of the presence of a malignant tumor, and the difference in SPR indexes for blood cells with verapamil taken before and after glioma resection can serve as a marker of completeness of tumor removal. Specifically, if the SPR index of the blood cells with verapamil after the surgery is lower than before it, the conclusion about the complete tumor removal can be made. In the opposite case (the SPR index is higher), the tumor removal is incomplete (Fig. 3b). This allows for the assessment of the completeness of malignant tumor resection based on blood cell aggregation level. The reliability of the conclusions ascertained from the analysis of SPR index changes was confirmed by other tumor imaging methods, namely magnetic resonance imaging and computed tomography [16].

In this study, the completeness of malignant tumor resection was investigated for 12 patients with grade II gliomas (Table).

As mentioned above, a 0.25% solution of verapamil hydrochloride, diluted 10,000 times, was used in the analysis. For all patients, before the surgery, the SPR index of blood cells with added verapamil hydrochloride was larger compared to that of blood cells with water only. After tumor removal surgery, 7 patients showed a smaller SPR index of blood cells with added verapamil hydrochloride compared to pre-surgery values, indicating complete tumor resection. However, for 5 other patients,

Table. SPR indices of blood cells with the addition of verapamil hydrochloride for patients with brain glioma, measured before surgery and 7 days afterward.

No	SPR indices for patients		Behavior of SPR index	Conclusion
	Before surgery, degree	After surgery, degree		
1	2.0971	1.3913	SPR index decrease	Complete resection
2	1.8236	1.445		
3	1.2178	1.1832		
4	0.9717	0.8462		
5	1.3524	1.1716		
6	0.9223	0.8251		
7	1.4282	1.1529		
8	1.0609	1.2020	SPR index increase	Incomplete resection. Prevention of tumor progression is necessary.
9	1.1529	1.4282		
10	0.9275	1.5195		
11	2.2683	2.4715		
12	1.4282	2.4282		

an increase in the SPR index was observed, indicating incomplete tumor resection.

Thus, the developed integrated sensor system based on the phenomenon of SPR allows detecting of small tumor remnants due to its ability to register the changes in the aggregation of peripheral blood cells caused by micro-inflammation in the place of malignant brain tumors. This is particularly relevant for grade II malignant tumors for which an inflammation related to the growth of residual tumor tissue is not always detected by conventional laboratory tests [17].

The proposed approach differs from other methods in its accessibility and quick obtaining of SPR indices, it does not require expensive reagents or radioactive labels. Additionally, the method can be further developed for other types of tumors, as it is universal, unlike tissue-specific oncological markers [18–20]. Measuring changes in SPR indexes provides a more refined tool for detecting the presence of tumor tissue during the postoperative period, enabling early preventive measures to be taken against tumor progression. Furthermore, this method allows tumor screening at any time after surgery.

4. Conclusions

An integrated sensor system based on the SPR phenomenon has been developed for early diagnosis of the postoperative progression of malignant brain tumors. The key components of this system are the "Plasmon-6" biosensor device (model 321), a thermal box, a plunger pump, and an air dryer. The system application has been demonstrated for assessing the completeness of resection of grade II malignant brain glioma by analyzing blood cell aggregation level. The proposed sensory system and diagnostic method based on the SPR phenomenon allow detecting of incomplete brain tumor resections and early postoperative preventing of tumor progression.

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Інтегрована сенсорна система на основі явища поверхневого плазмонного резонансу для ранньої діагностики післяопераційного прогресування злоякісних пухлин мозку

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Анотація. У роботі створено інтегровану сенсорну систему на основі явища поверхневого плазмонного резонансу (ППР), яка складається з біосенсорного приладу “Плазмон-6” (модель 321), плунжерного насоса та осушувача повітря, розташованих у термобоксі. Стабілізація температури чутливого елемента біосенсора особливо важлива для дослідження біологічних середовищ. Систему застосовано для оцінки повноти резекції злоякісної пухлини головного мозку II ступеню за зміною рівня агрегації клітин периферичної крові змішаної з розчином верапаміл гідрохлориду. З цією метою для 12 пацієнтів порівнювались показники ППР для клітин крові, взятої перед операцією та на сьомий день після неї. Зниження значень показників ППР після операції порівняно з передопераційними даними вказує на повну резекцію пухлини пацієнта, а зростання показників ППР – на наявність залишків пухлини та пов’язаного з ними мікрозапалення, а отже, на неповну резекцію пухлини та ймовірність рецидиву через прогресування пухлини. Показано, що зазначене в протоколі операції повне видалення гліоми головного мозку було у 7 пацієнтів, а у 5 пацієнтів – неповне. Перевагами використання запропонованої сенсорної системи для оцінки повноти видалення злоякісної пухлини є швидке отримання показників ППР, чутливість до малих доз речовини, безпека для пацієнтів, автономність і мобільність. Це дозволяє своєчасно вжити заходи для запобігання післяопераційного прогресування злоякісної пухлини.

Ключові слова: інтегрована сенсорна система, поверхневий плазмонний резонанс, плунжерний насос, осушувач повітря, гліома, повнота резекції пухлини.