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Реферати

КОНЦЕПТУАЛЬНІ ОСНОВИ ВІДНОВНОГО МОРФОГЕНЕЗУ ПЕЧІНКИ ПІД ВПЛИВОМ РАДОНОВОЇ БАЛЬНЕОТЕРАПІЇ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ АУТОІМУННОМУ ПОШКОДЖЕННІ

Єгорова Т. М.

Експеримент проведено на білих нелінійних статевозрілих щурах-самцях. Проводили лектиногістохімічні дослідження, електронно-мікроскопічні, морфометричні та метод статистичного аналізу. Експериментально доведено саногенетичну дієвість радонової бальнеотерапії аутоімунного пошкодження печінки як аналога аутоімунного гепатиту, а саме – гальмування лімфоцитарної аутоагресії та її наслідків, і особливо, прояви активного відновного морфогенезу завдяки адаптогенним та імуномодуляторним (з посиленням супресорної ланки) властивостям радону.

Ключові слова: гепатоцити, лімфоцитарна аутоагресія, радонотерапія, відновний морфогенез.

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КОНЦЕПТУАЛЬНЫЕ ОСНОВЫ ВОССТАНОВИТЕЛЬНОГО МОРФОГЕНЕЗА ПЕЧЕНИ ПОД ДЕЙСТВИЕМ РАДОНОВОЙ БАЛЬНЕОТЕРАПИИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АУТОИММУННОМ ПОВРЕЖДЕНИИ

Егорова Т.Н.

Эксперимент проведен на белых нелинейных половозрелых крысах-самцах. Проводили лектиногистохимические, электронно-микроскопические и морфометрические исследования, а также использовали методы статистического анализа. Экспериментально доказано саногенетическое влияние радоновой бальнеотерапии аутоиммунного повреждения печени как аналога аутоиммунного гепатита, а именно – торможение лимфоцитарной аутоагрессии и ее последствий, особенно проявления активного восстановительного морфогенеза, благодаря адаптогенным и иммунномодуляторным (с усилением супрессорного звена) свойствам радона.

Ключевые слова: гепатоциты, лимфоцитарная аутоагрессия, радонотерапия, восстановительный морфогенез.

Рецензент Волков К.С.

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COULOMB INTERACTIONS AT THE SILICON WIRE-NERVOUS TISSUE INTERFACE

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In the past decade the “silicon crystal-nervous tissue” interface has been attracting huge interest due to the possibility of its application for neuro-computing and regenerative medicine. The key challenge of this research is to understand the mechanisms of the interface formation, and thus to prespecify properties of the interface and its operation. Despite numerous researches there is no clear definition of these mechanisms up to date. Here we present the study of the “silicon wire-nerve tissue” interface formed both in vivo and in vitro experiments. We have shown experimentally that there is a very good electrostatic adhesion of a nerve tissue to silicon wire in the living organism and in a medium, close to the physiological environment, as well. Strong interaction between the constituents of the interface was found to result from Coulomb mutual attraction of the oppositely charged surfaces of the nerve fiber and silicon wire. We measured the surface density of the charge at both surfaces of the interface using dual-gated SOI-nanotransistors. The surface density of positive charge at the nerve membrane and the surface density of the negative charge at the silicon wire were found to be ~$2x10^{13}$ cm^{-2} and ~$1x10^{14}cm^{-2}$, respectively. We analyzed Coulomb interactions at the interface during propagation of a nerve impulse and concluded that nerve impulse has to initiate a flexural wave in the nerve fiber and to generate an electronic surfacial wave in a space charge region of silicon wire. Moreover, the flexural wave has to provide metabolism in the nerve fiber and, hereby, vital capacity of the interface. On the other hand, the electronic wave in the space-charge region of silicon wire allows using it for extracellular recording of neuronal signal.

The interface of living-nonliving matter has become a subject of research in many fields of science. Study of the restoration of normal activity of an injured or diseased human nervous system attracts special attention. Intensive research in this area became more active several years ago [1-6]. It is evident that integration of living organisms with functional materials can create intelligent nano- and

macro-scale living complexes for biotechnological and medical practice. In this regard silicon wires are the most suitable matter for several reasons. Firstly, silicon is a biocompatible material. It may be naturally extracted from the living organism due to its easy oxidation, while permanent implants of silicon wire may be done too using a special treatment of the silicon surface. Furthermore, silicon wires were found to stimulate growth of the newly formed nerve tissue [7-9]. In addition, the study of the "silicon wire-nerve tissue" interface has been enhanced within the past years due to development of the silicon based nano- and neuro-computers [10-11], which might replace a damaged part of the brain and ensure their joint operation with a healthy part of nervous system. The key problem of all this research is "know how" to form such complexes with prespecified properties and to ensure control over their activity.

The goal of the current work is to study the mechanism of the "silicon wire-nervous tissue" interface formation. For this purpose we prepared specimens of the "silicon wire-nerve tissue" interface in experiments carried out in vivo by simulation of a sciatic nerve injury in rats and subsequent recovery of the nerve tissue by using an implant comprising silicon nano- and micro-wires. The silicon wires used for preparation of the implants were grown by gas-phase-reactions method in a sealed tube at a temperature gradient [12, 13]. Just before surgical operation, a surface cleaning of the prepared set of nano- and micro-wires was done. Thereupon, the wires were oxidized by storage under ambient atmosphere at room temperature. The thickness of the oxide does not exceed one to two nanometers. Surgical operations and preparations of slices of the interface are described elsewhere [8]. Morphology of the interfaces was examined by light microscopes Carl Zeiss NU-2E and Olympus BX 51 and transmission electron microscope TEM-125K, (SELMI, Ukraine).

To understand details of the interface formation, we performed additional experiments in vitro. In this experiment, we used the silicon nanowire field effect transistor (SiNW-FET) as the sensor element to clarify all stages of the interface formation. The experiment in vitro was carried out on a living neuron immersed into a physiological environment.

The interfaces prepared in vivo were examined in various postoperation periods ranging from 3 weeks up to 12 months. The growing nerve fibers formed within the short period were unmyelinated, while the others had myelin sheath whose thickness depended on the length of the postoperation period. Typical micrographs of the "silicon wire-nerve fiber" interfaces made with the light and transmission electron microscopes are shown in Figs 1a and 1b, respectively.

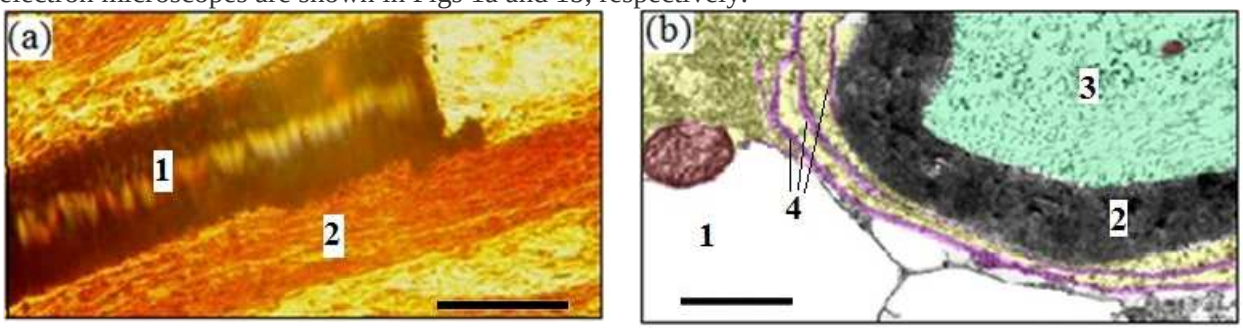

FIG.1. Micrographs of the interfaces: (a) made with light microscope, the slice is impregnated with nitric silver, plane of the slice coincides with the long axis of the silicon wire; here 1 is the silicon wire, 2 is a bundle of the newly formed nerve fibers; (b) made with transmission electron microscope, the slice treated with 1% water solution of osmic acid, plane of the slice was perpendicular to the long axis of the silicon wire; here 1 is the silicon wire, 2 is myelin sheath, 3 is axoplasm, 4 is new layers of the myelin sheath formed of Schwann cells. Scale bar: 40 μm – (a), 50 nm – (b).

Before examining the micrographs, it is worthy to note specific features of slices preparation that is induced by high difference in mechanical strength of the nerve fiber and the silicon wire, even though the slices were made at deep freezing conditions. We prepared slices of two orientations, i.e. along and normal to the large axis of the wires. Examination of a great number of slices revealed breaking of the silicon wire which had occurred in the course of their preparation. Sometimes, a part of the crystal either retained or failed, but biomaterial conserved. Examination of the interface with different magnifications allowed seeing general picture of the growing nerve fibers in the vicinity of the silicon wire and a set of various cells supporting growth of the nerve. In Fig. 1a a micrograph of the interface made with light microscope demonstrates how a bundle of the newly grown young nerve fibers tightly adhere to the silicon wire. The micrograph of the interface made with transmission electron microscope (Fig. 1b) shows how the newly formed layers of cell membrane of regenerating nerve fiber adhere to silicon crystal. The distance between membrane and silicon wire is less than a few nanometers. Having analyzed a great number of micrographs, we can conclude that young regenerating nerve fibers adhere **to the**

surface of silicon crystals. Furthermore, **membrane,** which covers the nerve fiber, is **adjacent immediately** to silicon crystal. To understand sensitivity of the nerve fiber to silicon wire, we have to consider composition and the energy state of both constituents of the interface. The energy state of the near-surface region of the silicon wire at room atmosphere is shown in Fig. 2a. In our experiment, we used silicon wires doped by boron that means that position of the Fermi level in the bulk of the crystal $\mathbf{E_F}$ is placed nearby the top of the valence band.

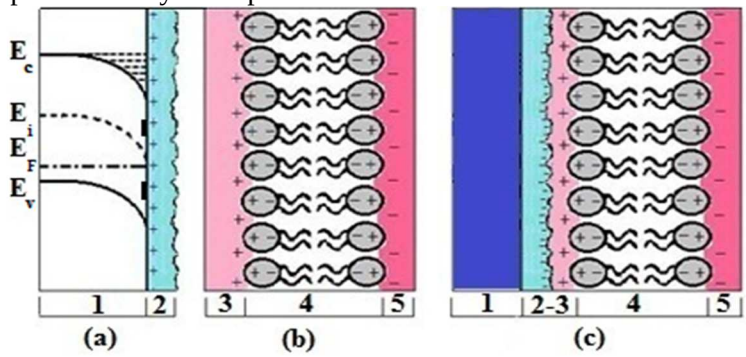

Fig. 2. (a) The near-surface region of silicon wire, where: 1– the energy structure of the near-surface region of the silicon wire; 2 – a native oxide layer on the wire surface. (b) A structure of the membrane of nerve fiber (axon) in the living organism, where: 3 – the extracellular physiological environment; 4 – the axon membrane composed of phospholipids molecules; 5 – the axoplasm. (c) A morphology of the "silicon wire – nervous tissue" interface generated in the living organism, where: 1 – silicon wire, 2-3 – the interface of a negatively charged native oxide and positively charged outer surface of the nerve fiber membrane, 4 – the axon membrane, 5 – the axoplasm.

A specific lattice restructuring of a few external atomic layers proper to the silicon surface is known [14] to initiate two energy bands located immediately at the surface. Density of the states in each of these bands is very high and approach density of atoms at the surface (~ 10^{14} cm^{-2}), therefore the Fermi level at the surface is placed near the middle of the energy gap $\mathbf{E_i}$ and its position slightly depends on doping [15] and growth of a thin native oxide as well [16]. However, in p-type of silicon, which is used in our experiment, a positive charge at the surfacial bands exceeds the negative one [15]**.**

Thus, the silicon wire being at vacuum or covered by the thin native oxide is entirely neutral, though the external surface of the silicon wire is charged positively. Structure of the nerve fiber membrane inside a living organism is shown in Fig. 2b. In our case preparation of the interface from the sciatic nerve of rats, the axon membrane is composed of phospholipid molecules that are known [17] to consist of polar heads and nonpolar tails and form the membrane in a shape of bilayer. It is worthwhile to emphasize that the outer side of the membrane is charged positively. Surface density of this charge, according to Richardson' structure model [18] equals about $2x10^{13}$ cm^{-2}. So, a large positive charge of about $2x10^{13}$ cm^{-2} is permanently located at the outer side of the membrane.

Summarizing the above consideration, we can draw the following conclusion. If the near-surface region of the silicon wire conserves its charge state inside the living organism, then the silicon wire and the nerve fiber are similarly charged and have to repulse each other. Nevertheless, we do observe a strong adherence of the nerve fiber to the silicon wire that allows supposing that the physiological environment (interstitial fluid, cell cytoplasm, etc.) contributes to the formation of the interface. Analyzing how the environment may influence the charge state of silicon wire, we paid attention to the main properties of the physiological environment. About 80% of the environment consists of water, and its pH equals about 7. On the other hand, thin native oxide layer, that covers the wires, is known [19] to consist primarily of intermediate oxidation states of Si atoms, in particular, Si^{1+} (Si_2O), Si^{2+} (SiO), Si^{3+} (Si_2O_3). Thus, we can suppose that sub-oxidized Si atoms chemically react with OH^- radicals of the environment, charge the surface of the wire negatively and, thereby, provide Coulomb attraction between silicon wire and nerve fiber. To validate this assumption, we used a model experiment on contact of the nerve cells with silicon wire in the electrolyte with pH = 7, close to the physiological environment. In this experiment SiNW-FET based on SOI-structure with two gates [20-22] has been used as the sensor element to evaluate charge states of the silicon wire during the interface formation. A schematic representation of this transistor and its optical image after adherence of the nerve cell are shown in Figs 3a and 3b. In this transistor, the substrate is used as a control gate (back-gate, BG), modulating their conductivity. An analyte which adheres to the free surface of the transistor plays the role of the second gate (virtual local gate). If the charge at the surface of the nanotransistor changes due to adsorption of the analyte, so will change the conductivity of the nanotransistor and will shift its current-voltage $\mathbf{I_{ds}(V_{bg})}$ characteristic along voltage axis. A sign and value of the shifting allow determining both the sign and the density of the adsorbed charge. To elucidate how the charge state of the nanotransistor surface changes in contact with the physiological environment and after adherence of a neuron, we studied current-voltage characteristics $\mathbf{I_{ds}(V_{bg})}$ in three cases: (1) initial state of the surface of the nanotransistor (without any analyte, i.e. a free surface covered with native oxide only), (2) the surface of the nanotransistor in a

contact with the physiological environment, and (3) the surface of the nanotransistor after adherence of a neuron when it is immersed into the physiological environment.

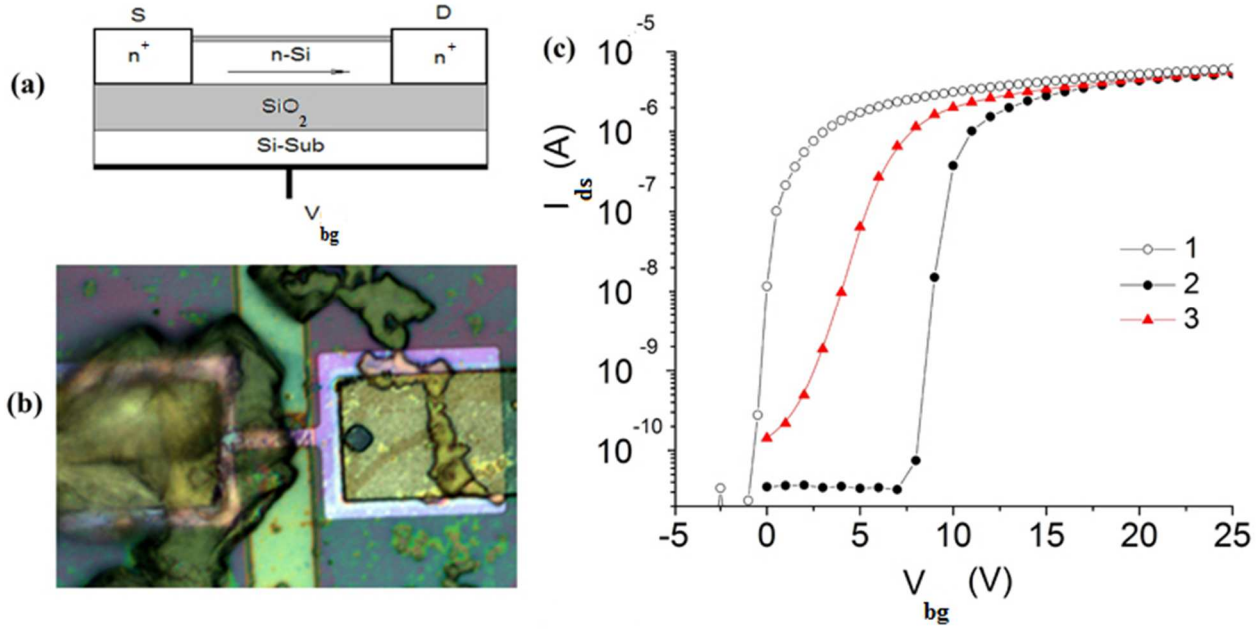

FIG. 3. Schematic presentation a dual-gated SiNW-FET biosensor based on SOI-structure (a); micrograph of a neuron adhering to the surface of biosensor (b); current-voltage characteristics $I_{ds}(V_{bg})$ for three cases of the surface of biosensor (c): 1 is the surface covered by native oxide (without any analyte), 2 - the surface in contact with the physiological environment, 3 - the surface in contact with adsorbed neuron that was immersed into the physiological environment.

The current-voltage characteristics for these three cases are shown in Fig. 3c. It is seen that when we immerse the nanotransistor into the physiological environment, the current-voltage characteristics shifts to the greater voltage $\mathbf{V_{bg}}$ that corresponds, by conditions of our experiment, to a negative charging of the surface of the nanotransistor. Then, we immerse a neuron into the physiological environment and observe its adherence to the surface of the nanotransistor (Fig. 3b). The adherence of the neuron is accompanied by shifting of the current-voltage characteristic in the opposite direction, in particular, to the smaller voltage $\mathbf{V_{bg}}$, that means an accumulation of a positive charge at the surface of the nanotransistor. Knowledge of the shifting of the current-voltage characteristics and geometric parameters of the nanotransistor allows calculating the surfacial charge at the surface of the nanotransistor induced by the adherence of the analyte. We calculated the surface density of this charge after adherence of components of the physiological environment and after adherence of a neuron as well. We found that the charge accumulated in physiological environment on the surface of the silicon nanotransistor is **negative** and its density equals $\sim 1 \times 10^{14} cm^{-2}$. On the other hand, the adsorption of a neuron initiates accumulation of a **positive charge** on the surface of nanotransistor**.** The density of this charge is equal to $\sim 2 \times 10^{13}\ cm^{-2}$.

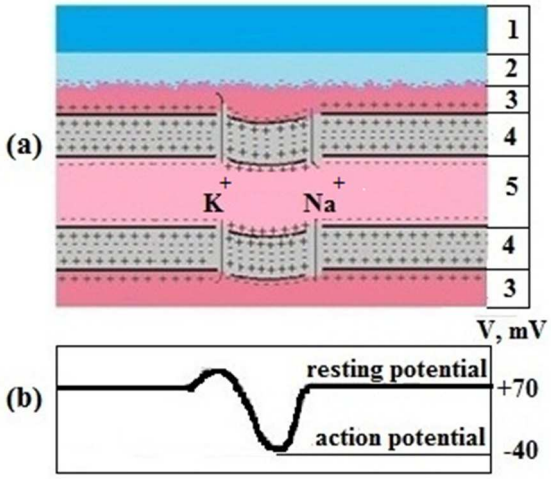

Fig. 4. Charge state of the interface (a) during a nerve impulse (b) propagation. Here 1 is silicon wire, 2 is native oxide with negative charge on its surface, 3 is the extracellular physiological environment, 4 is the axon membrane, 5 is the axoplasm.

So, the experiment in vitro proved the above made assumption about chemical reaction of native oxide with OH^- radicals and, hereby, negatively charging a surface of the native oxide of silicon wire. Furthermore, the density of the positive charge accumulated at the silicon-nanotransistor after adsorption of the neuron coincides with the known value of the density by Richardson' structure model [18]. So, from the in vitro experiment, we can draw a conclusion on the Coulomb origin of the interface formation and present morphology of the "silicon wire – nervous tissue" interface as it is shown in Fig. 2c. It is also evident that a propagation of the nerve impulse through the nerve fiber has to occur in a quite different way as compared to the case when the nerve impulse passes through a free nerve fiber. A charge state of the formed interface during propagation of nerve impulse schematically is shown in Fig. 4.

At a normal (resting) state of the nerve fiber, besides a permanent positive charge at the outer side of the membrane, there is an additional positive charge located inside the extracellular medium and the negative charge located inside the axoplasm. These charges produce potential difference across the axon membrane, the so called "resting potential" (V_{rest} ~ 70 mV) that acts throughout the entire length of the nerve fiber in a normal (resting) state of the nerve. However, when a nerve impulse passes along the nerve fiber, it reverses the potential difference across the axon membrane, the so called, "action potential" (V_{action} ~ - 40 mV). So, propagation of the nerve impulse along the nerve fiber has to be accompanied by a flexural wave in the nerve due to recharge of the external side of the membrane and subsequent changing of the Coulomb attraction of the nerve fiber to the silicon wire by the Coulomb repulsion. Additionally, propagation of the nerve impulse has to generate an electronic surfacial wave in a space charge region of the silicon wire. The latter may be used for extracellular recording of neuronal signal. Details of this process have to depend strongly on properties of silicon wires and call for further research.

Summary

We carried out an experimental research on the formation of the interface "silicon wire - nervous tissue". We analyzed an intermolecular mechanism of adherence of nerve tissue (nerve fiber, neuron) to silicon wire from the physico-chemical point of view. We presented detailed structure of the interface and have experimentally shown that the main force of the stable interface formation is related to the Coulomb mutual attraction of oppositely charged surfaces of the nerve tissue and silicon crystal. We also paid attention to specifics of the nerve impulse propagation along the nerve fiber and showed that the Coulomb interaction of the nerve fiber and the silicon wire has to be accompanied by two kinds of the waves, in particularly, a flexural wave in the nerve fiber and an electronic surfacial wave in a space charge region of the silicon wire. Stimulation of a flexural wave has to provide metabolism in the nerve fiber and, hereby, vital capacity of the interface. On the other hand, the electronic wave in the space-charge region of silicon wire allows using it for extracellular recording of neuronal signal. Thus, the presented experimental results demonstrate how to ensure control of the interface properties for its application both in neuro-computing and medical practice.

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Реферати

КУЛОНІВСЬКІ ВЗАЄМОДІЇ НА ИНТЕРФЕЙСІ «НИТКОПОДІБНИЙ КРИСТАЛ КРЕМНІЮ-НЕРВОВА ТКАНИНА»

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В останні роки інтерфейс «ниткоподібний кристал кремнію-нервова тканина» привертає особливу увагу в зв'язку з тим, що кремній є найперспективнішим матеріалом для розробки нейро-комп'ютерів і для регенерації нервової тканини. Головне завдання цих досліджень - з'ясування механізмів формування таких інтерфейсів і розробка методів управління їх властивостями. Незважаючи на безліч публікацій, до теперішнього часу немає чіткого розуміння механізмів формування цих інтерфейсів. Відсутність знань є головною перешкодою на шляху створення пристроїв, що регенерують пошкоджену нервову тканину, або пристроїв, здатних повністю замінити її пошкоджені ділянки і злагоджено працювати зі здоровою частиною нервової системи. У цій роботі ми представляємо результати досліджень інтерфейсу «ниткоподібний кристал кремнію-нервова тканина» в експериментах *in vivo* та *in vitro*. Експерименти показали, що адгезія нервової тканини до ниткоподібних кристалів кремнію як в живому організмі, так і в фізіологічному середовищі, пов'язана з електростатичною взаємодією між поверхнями обох складових інтерфейсу. Сильна взаємодія компонентів інтерфейсу обумовлена кулонівською взаємодією протилежно заряджених поверхонь нервового волокна і кремнієвого ниткоподібного кристалу. Це було показано експериментально з використанням кремнієвого польового нанотранзистора з двома затворами. В цьому експерименті ми визначили знаки і виміряли густину заряду на обох поверхнях інтерфейсу. Виявилося, що поверхня мембрани нерва заряджена позитивно і густина заряду дорівнює ~ $2x10^{13}$ $см^{-2}$, поверхня кристала кремнію – заряджена негативно, і густина заряду дорівнює ~ $1x10^{14}cm^{-2}$. З огляду на кулонівську взаємодію між нервовим волокном і кристалом кремнію, ми можемо зробити висновок про те, що проходження нервового імпульсу по нервовому волокну, яке супроводжується зміною знака заряду на поверхні волокна, має призводити до появи згинальної хвилі в нерві і до генерації поверхневої електронної хвилі в шарі просторового заряду в ниткоподібному кристалі кремнію. Виникнення цих хвиль має приводити до двох позитивних ефектів, а саме, згинальна хвиля повинна покращувати метаболізм в нервовій тканині, і таким чином, забезпечувати життєздатність інтерфейсу, а поверхнева електронна хвиля, що генерується в ниткоподібному кристалі кремнію, може бути використана для неінвазивного способу реєстрації нервових імпульсів.

Ключові слова: інтерфейс, ниткоподібний кристал кремнію, нервова тканина.

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В последние годы интерфейс «нитевидный кристалл кремния-нервная ткань» привлекает особое внимание в связи с тем, что кремний является самым перспективным материалом для разработки нейро-компьютеров и для регенерации нервной ткани. Главная задача этих исследований это выяснение механизмов формирования таких интерфейсов и разработка методов управления их свойствами. Несмотря на множество публикаций до настоящего времени нет четкого понимания механизмов формирования этих интерфейсов. Отсутствие этих знаний является главным препятствием на пути создания устройств, регенерирующих поврежденную нервную ткань, или устройств, способных полностью заменить ее поврежденные участки и согласованно работать со здоровой частью нервной системы. В настоящей работе мы представляем результаты исследований интерфейса «нитевидный кристалл кремния-нервная ткань» в экспериментах *in vivo* и *in vitro*. Эксперименты показали, что адгезия нервной ткани к нитевидному кристаллу кремния как в живом организме, так и в физиологической среде связана с электростатическим взаимодействием между поверхностями обеих составляющих интерфейса. Сильное взаимодействие компонентов интерфейса обусловлено кулоновским взаимным притяжением противоположно заряженных поверхностей нервного волокна и кремниевого нитевидного кристалла. Это было показано экспериментально с использованием кремниевого полевого нанотранзистора с двумя затворами. В этом эксперименте мы определили знаки и измерили плотности заряда на обеих поверхностях интерфейса. Оказалось, что поверхность мембраны нерва заряжена положительно и плотность заряда равна ~ $2x10^{13}$ $см^{-2}$, поверхность кристалла кремния – заряжена отрицательно и плотность заряда равна ~$1x10^{14}cm^{-2}$. Учитывая кулоновское взаимодействие между нервным волокном и кристаллом кремния, мы можем сделать вывод о том, что прохождение нервного импульса по нервному волокну, которое сопровождается изменением знака заряда на поверхности волокна, должно приводить к появлению изгибной волны в нерве и к генерации поверхностной электронной волны в слое пространственного заряда в нитевидном кристалле кремния. Возникновение этих волн должно приводить к двум положительным эффектам, а именно, изгибная волна должна улучшать метаболизм в нервной ткани, и таким образом, обеспечивать жизнеспособность интерфейса, а поверхностная электронная волна, генерируемая в нитевидном кристалле кремния, может быть использована для неинвазивного способа регистрации нервных импульсов.

Ключевые слова: интерфейс, нитевидный кристалл кремния, нервная ткань.

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