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Morphology of the interface “silicon wire – nerve fiber"

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Abstract. In the last few years, a special interest was attracted to research of the interface “silicon wire - nerve tissue", due to opening up possibilities to use it to recover nervous system after traumatic damages or damages induced by disease. A key problem of this research is a lack of “know how” to test the physicochemical state of the interface and to control its properties. Although, a lot of work in this field was performed, no detailed description of the interface formation from the surface physics point of view has been provided so far. In the current research, we prepared specimens of the interface “silicon wire - nerve fiber". Experiments were carried out in vivo. We examined the morphology of the interface and the mechanisms of adhesion in more detail and proposed a physical model of intermolecular interaction of silicon surface and nerve fiber.

Introduction

Despite the enormous advances in the field of neurosurgery, nerve damage still remains one of the large-scale problems which affects about one million people worldwide [1]. The most severe traumatic injuries of the extremities, such as crush and tear lead to various neuropathies and lack of motor function. And, of course, their recovery is possible only with microsurgical interventions [2]. In this regard, there exists a need for a transplant, which would not only be filled in the missing portions of the nerve, but also would affect the regeneration process.

One of the most effective methods to recover damaged nerves is microsurgical autotransplantation when the graft is made from one part of the body to another part of the same organism. This method, when applied in a short period of time after the injury, restores well the injured organ. However, it has several drawbacks. The method is applicable only to restore the short nerve gaps [3, 4] and is accompanied by a loss of functionality of the donor area of the body. Recovery of nerves is accompanied by the need of two or more operations, which increases the duration of the surgical intervention and reduces the efficiency of the functional recovery of the working body.

There are several other techniques for the insertion into the injured nerve gap region of various organic and inorganic materials in the form of hollow tubular structures [5] with the primary purpose to direct the growth of new nerve fibers from the proximal to the distal end [6]. The use of implants of conductive materials, such as metals or semiconductors, can both support the ordered growth of nerve fibers and transit nerve signals. Furthermore, the conductive materials allow electrostimulation of nerve tissue regeneration [7]. In this regard, silicon wires are the most challenging matter by several reasons. Firstly, silicon is a biocompatible material. Further, it is extracting from the living organism by the native way, in contrast to metal implants [8]. Then, at a causal necessity permanent implants of silicon may be done too, for instance, by using a special treatment of the silicon surface. At last, the interface of nerve tissue with silicon nano- and micro-crystals allows the development and application of nano- and neuro-computers in living organisms

[9 -11], which can replace a damaged part of nervous system and operate with the remaining healthy part of nerve tissue. The key problem of all this research is to understand the physicochemical state of the interface and to develop methods to obtain the prespecified properties. In the future, this knowledge will help solving a number of complex problems in the field of a treatment and will prolong the life time of an intact human nerve system.

In the current research, we prepared specimens of the interface “silicon wire -- nervous fiber" in experiments carried out in vivo by simulation of a sciatic nerve injury. One of the techniques, which were used in our work for the interface “silicon wire - nerve fiber" preparation, is described below. It includes several stages: growth of silicon wires; handling the implants; surgical procedure; and preparation of slices of the interface. Examination of the morphology of the interface shows that a strong adhesion of nerve tissue to silicon wire is caused by Coulomb interaction.

Experimental procedure

Growth of silicon wires. Silicon wires (see Fig. 1) were grown by the technology developed by Sandulova et al. [12]. This technology is based on a method of gas-phase-reaction in a sealed tube at a temperature gradient. In order to provide the chemical reactions and to stimulate rapid growth of the wires, we used bromine and gold. For growing wires with a prespecified type and value of conductivity, we added doping impurities into hot part of the tube. Due to differences in the reaction binding energies of gold and the doping impurities with bromine, the temperature gradient provides a different amount of precipitation of these materials along the tube. That is why, grown silicon wires are distributed along the tube by size (diameter, length) and by the level of doping [13]. The thinner the diameter of a wire is, the smaller is the concentration of dopants. The diameter of the grown wires ranges from 10 nm to several tens of microns. Their length varies in a range from tens of microns up to a few centimeters. Furthermore, the shape of wires depends on their diameter, too. The wires, which diameter was of nanometers, were cylindrical, while the wires much greater diameters were hexahedral.

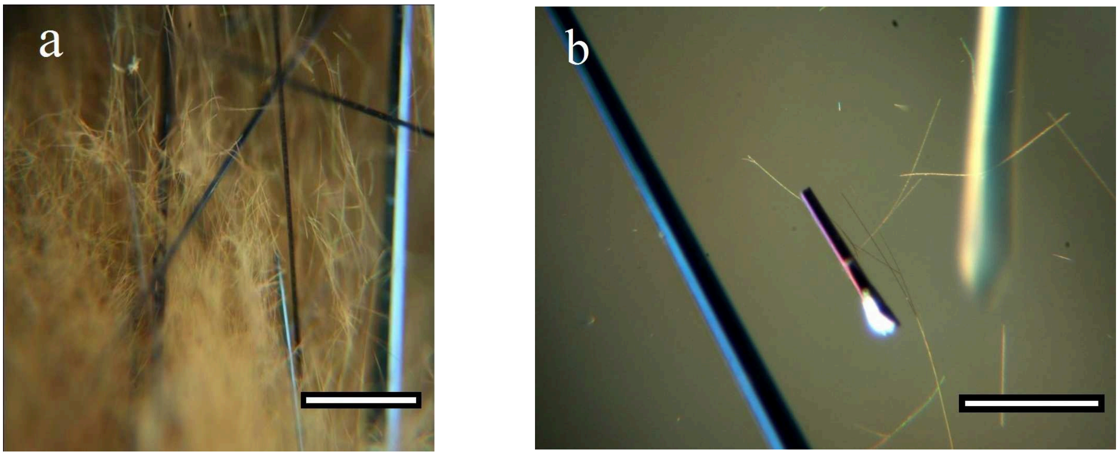

Fig. 1. Wires for preparation of implants. Scale bar is 80 μm (a), 250 μm (b)

Handling the implants. Making of implant started from dividing the wires by diameters. Wires for preparation of implants are shown in Fig. 1. The prepared set of nano- and micro-wires was treated for purification of a surface in different etchants. Thereupon, the wires were oxidized by storage under ambient atmosphere at room temperature. The thickness of silicon oxide does not exceed one to two nanometers. Just before surgical operation, an antispiking gel (Mezogel, gel against adhesions absorbable, 50 ml, Linteks, St. Petersburg, 9393-009-56257679-2010, № 2010/08895 21.09.2010) was introduced into the aorta extracted from another rat. In order to avoid a rejection of the transplant, the aorta has been pre-frozen in liquid nitrogen. Then, the set of the wires was placed into the gel and oriented along an axis of the aorta.

Surgical procedure. Experiments were performed by standard procedure on several groups of adult rats of Wistar line. To exclude a miss operation, animals were divided into three groups. For the animals of the first group after sodium thiopental anesthesia (60 mg / kg) and dissection of sciatic nerve we insert the implant. The second group of animals was used for simulating trauma of the sciatic nerve without the use of the implant. The third group was composed of sham-operated animals. Details of surgical operation and estimation of functionality of legs in the postoperation period are described elsewhere [9].

Preparation of slices of the interface. Three weeks after the operation, animals of the first group were taken out of the experiment by decapitation with use an overdose of thiopental anesthesia. Nerves with the implant were extracted, and slices were produced using a cryotome (MK-25, “Tekhnolog” Russia). Thereupon, the slices were stored during a day in 10% neutral formalin, next rinsed in distilled water and fixed on a microscope slide. For the purposes of microscope investigation of the nerve fibers, samples were stained with silver nitrate [14]. Prepared slices of the interface “nerve fiber - silicon wire" were examined by light microscopes Carl Zeiss NU-2E and Olympus BX 51 equipped with a digital camera.

Care for animals, labeling and all manipulations were carried out under the provisions of the "Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes".

Results and Discussion

Micrographs of the interfaces “nerve fiber-silicon wire” are presented in Fig. 2.

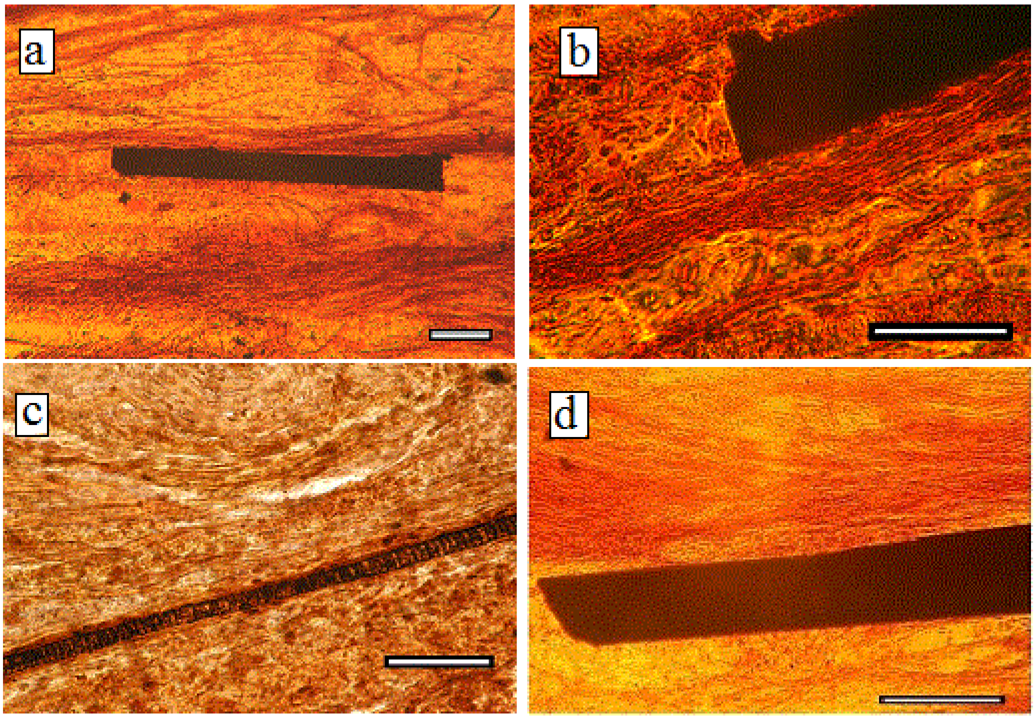

Fig. 2. Micrographs of the affected nerve with the implanted silicon wires. Scale bar is: (a) – 150 µm, (b) – 50 µm, (c) – 60 µm, (d) - 80 µm.

Before analyzing the micrographs, it is worthy to point out a specification of the preparation of the slices that is induced high difference in mechanical strength of nerve fiber and silicon wire. We attempted to prepare all the slices oriented primarily along the large axis of the wires. High deviation from this direction resulted in breaking off and falling out a piece of the crystal and in a persistence of a mark of the crystal removed part as a residual of the biomaterial. This may be seen in the micrographs of Figs 2 a and 2 b. Slight deviation from this direction resulted in persistence of beveled cut of biomaterial placed on the crystal surface. In case, if the persistent layer of biomaterial is sufficiently thin, then one can see crystals, which accrete from every side by arrays of regenerating nerve fibers. In another case, interface "nerve fiber - silicon wire" is clearly seen along all length of the wire (see Fig. 2 c). High sensitivity of the nerve fibers to silicon wires is clearly seen from Fig. 2 d, which presents how the array of growing nerve fibers changes a direction of their growth, when it meets the silicon wire, adsorbs on a surface of the wire and carries on further growth across the surface. In all these cases, though their variety, we can conclude on high sensitivity growing nerve to the surface of silicon crystals. Summarizing results of an exam of the interfaces "nerve fiber-silicon wire", we may conclude that immediately on the surface of crystals there are arrays of young regenerating nerve fibers, which alternate with neogenic capillaries and collection of cells that support growth of the nerve.

To understand the affinity of the nerve fiber to the surface of the silicon wires found experimentally, we have to consider the composition and the energy state of both constituents of the interface, i.e. the silicon wire and the nerve fiber.

A sketch of a young regenerating nerve fiber (axon) is show in the left part of Fig. 3. An axon consists of axoplasma (2) covered by thin plasma membrane (1). In our case, preparation of interface from sciatic nerve of rat, plasma membrane is composed of phospholipids in a shape of bilayer [15]. In a normal state of the axon, there is a charge (of Na^+ and K^+ ions) distribution that forms the "rest potential" of ~ 60 mV between the external and the inner side of the membrane. It is worthy to note that the *outer surface of the plasma membrane is charged positively*.

To understand the affinity of nerve fibers to the surface of the silicon wires found experimentally, we have to consider also what the crystal surface consists of. A sketch of the energy band structure of a silicon wire is shown in the right part of Fig. 3. The energy structure of the near-surface region of a silicon crystal (7) has some specifics that made it special among many other semiconductors. A distinct restructuring of a few external atomic layers initiates two energy bands located immediately at the surface [16]. The density of states in each of these bands is very high and approaches to the density of atoms at the surface (~ 10^{15} cm^{-2}), therefore the Fermi level at the surface $\boldsymbol{F_s}$ is located nearby the middle of the energy gap $\boldsymbol{E_i}$ and slightly depends on doping [17] and growing of a thin native oxide as well [18]. Nevertheless, in p-type silicon, which is used in our experiment, a positive charge at the surface bands exceeds the negative one. So, the *free surface of the wires is charged positively* (9)*, i.e., similarly to the outer surface of the plasma membrane.* It is evident that if the electronic state of the silicon wire in living organism, i.e., in physiological environment (7) persists the same, - the nerve fiber and silicon wire have to repulse each other.

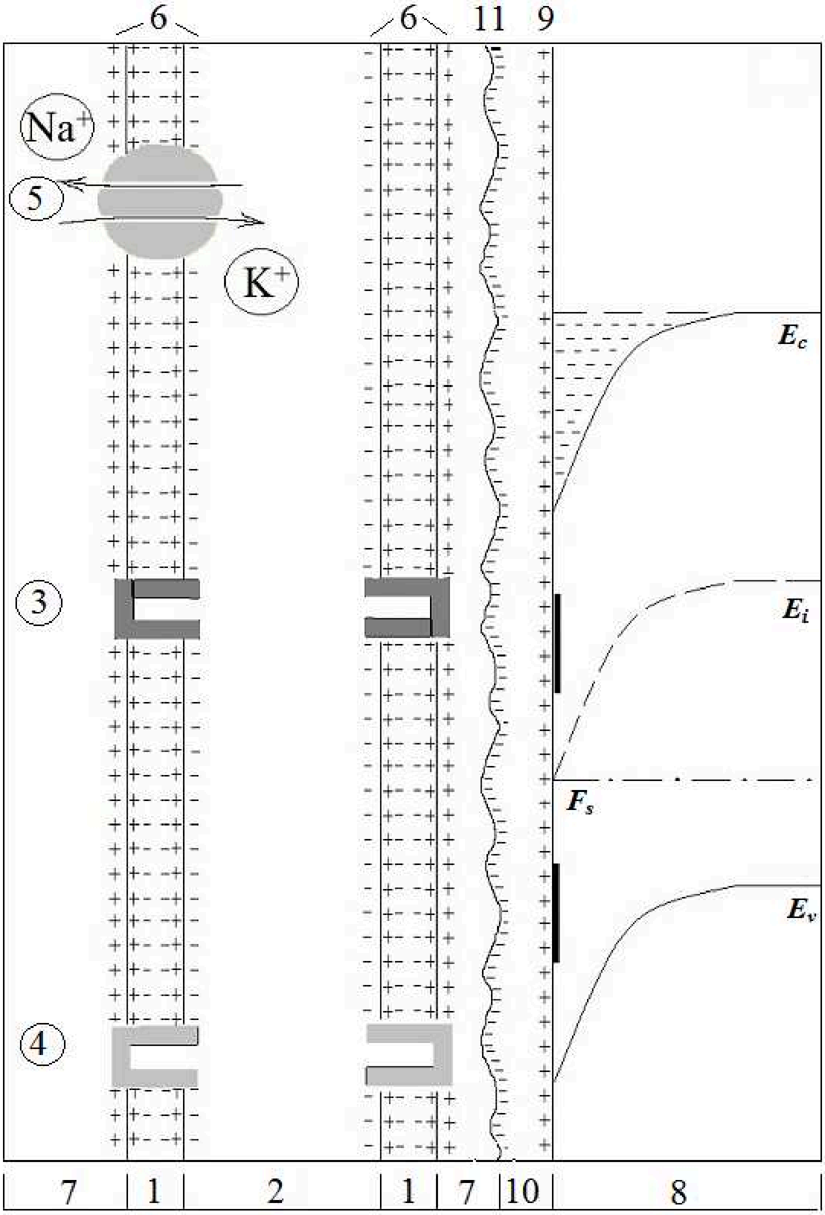

Fig. 3. Sketch of the morphology of the interface “silicon wire – nervous fiber" in the normal state of the nerve fiber (at the absence of the nerve impulse), where: 1 – plasma membrane; 2 – axoplasm; 3 – Na^+ channels and 4 – K^+ channels, permanently closed and opened only for exchange at the propagation of the nerve pulse; 5 – ion pump, which provides the exchange of K^+ and Na^+ ions between the axoplasm and ambient physiological environment; in the normal state ion pump supports the “rest potential” (~ -65 mV); 6 – charges at the outer and the inner sides of the axon membrane; 7 – ambient physiological environment; 8 – energy band structure of a near-surface region of the silicon wire; 9 – positive charge located at the near-surface bands; 10 – ultrathin native oxide layer, which consists primarily of intermediate oxidation states of Si atoms, in particular, Si^{1+} (Si_2O), Si^{2+} (SiO), Si^{3+} (Si_2O_3); 11 – negative charge located at the native oxide surface.

Let us consider how the physiological environment (7) may affect on the electronic state of a silicon wire. First of all, we paid attention to the content of the environment. The environment consists of about 80% of water, and its pH equals about 7. This means that in the physiological environment there are large amounts of OH^- radicals. On the other hand, the thin native oxide layer that covers the wires used, is known to consist primarily of sub oxidized Si atoms, in particular, Si^{1+} (Si_2O), Si^{2+} (SiO), Si^{3+} (Si_2O_3) [19]. Taking into account additionally that there is a positive charge at the near-surface bands of the silicon wire, we may suppose that sub oxidized Si atoms can create compound with OH^- radicals, charge the surface of the oxide layer negatively (11) and, thereby, provide Coulomb attraction of the nerve fiber to the silicon wire. This assumption has been proved experimentally by O.V. Naumova [20]. Thus, the Coulomb force is the main force that defines the interaction between nerve fiber and silicon wire.

Conclusion

We demonstrated a preparation technique of interface “silicon wire - nerve fiber" in experiments carried out in vivo. The examined morphology of the interface indicates that there is a high affinity of nerve tissue to silicon wire. It has been demonstrated that the adhesive properties of silicon wire arise from its interaction with the physiological environment. It has also been shown that the Coulomb force is the main force that defines the interaction between nerve fiber and silicon wire.

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