Biological verification of the long-range effect for silicon light irradiation for planaria

A V Stepanov1, D I Tetelbaum2, A I Dimitrieva1, A V Konstantinova1, D S Yumanov1, A P Popov1 and A V Kovalenko1

1Laboratory of Clinical and Hematological Research, Chuvash State Agricultural Academy, 29 Karl Marx Street, Cheboksary, 428003, Russian Federation
2Laboratory of Physics and Technology of Thin Films, Lobachevsky State University of Nizhny Novgorod, 23 Gagarina Avenue, Nizhniy Novgorod, 603950, Russian Federation

1E-mail: for.antonstep@gmail.com

Abstract. The effect of hypersonic waves excited by a heterostructure based on a silicon wafer with natural oxide on the survival of planaria after their decapitation has been experimentally investigated. The aim of the work was to study the physical and biological factors affecting the regeneration of planarians. The main object of study was a model organism – planaria Dugesia Tigrina. The planaria were decapitated, and then they were monitored by their habitat for a week. Identification and counting of microorganisms, bacterial microflora inoculation, temperature, and pH control were carried out. To explain the mechanisms that occur under the influence of hyperson, a mathematical model of the passage of hypersonic waves through a thin layer of water near the glass-liquid interface was developed. In the process of regeneration after decapitation of the experimental group, it was found that in the experimental group exposed to hyperson, the survival of planaria was 60% higher than in the control. It was shown that in an aqueous medium along the glass-water interface, hypersonic propagation occurs with less attenuation than in the water column. This leads to a waveguide effect and improved transmission of hypersonic effects to the studied organisms.

1. Introduction

Studies on the effect of millimeter-wave electromagnetic waves on living organisms, tissues and their use in medicine have led to the conclusion about their important functional role [1]. In this case, the generation and propagation of such waves is accompanied by acoustic vibrations and hypersonic waves, which serve as the active agent of this effect. The question remains open about the mechanism that provides the established anomalously large penetration depth of the exposure zone. It was previously established a phenomenon (long-range effect, LRE [2,3]) that can clarify this mechanism: when a solid is irradiated with light (using silicon as an example) in contact with a system containing an aqueous solution of NaCl (from 0.1 to 7%), there is a change in the properties of another sample located at a distance of several centimeters from the irradiated. According to the proposed model, the LRE is caused by the generation of hypersonic waves by the irradiated sample, and their propagation over long distances in this system is associated with the waveguide properties of the interface between the solution and the solid. These studies are interesting in that alkali metal ions are contained in living systems and are important components of biological processes.
In this paper, we study LRE in relation to living systems, namely to planar worms planaria (from the suborder Tricladida from the suborder of ciliary worms).

Planaria as an object of study are interesting in terms of their unique regenerative ability. So, regional human stem cells, which provide tissue regeneration processes in case of damage, are represented in organs by a very small fraction, which complicates their in vivo study. Unlike humans, adult stem cells of freshwater planaria, called neoblasts, account for up to 30% of the total number of planaria cells [4]. The conservative part of the human stem cell genome is homologous (about 90%) to the corresponding planarium genes [5, 6]. Some types of planarians (for example, Dendrocoelum lacteum) are not able to restore lost parts of the body by analogy with higher animals, but turning off a single gene (Dlac-β-catenin-1) recreates a regenerative ability that is unusual for this type of organism [7]. An interesting phenomenon is the influence of various influences on the regenerative process in planaria. So in [8], the influence of a weak magnetic field on the regeneration process of planarians was shown. In our work, we study the effect of hypersound on the regenerative abilities of planaria. Although there are results about planarians exposure under laser irradiation [9, 10] and low intensity LED irradiation [11], they are still not investigated at hypersonic waves.

The aim of this study is to verify the biological effects of the long-range effect consisting in the abnormally far propagation of hypersonic waves along the water-glass interface.

2. Materials and methods

Planaria of the species Dugesia Tigrina was selected as a model organism due to its unique ability to regenerate due to the high activity of stem cells [12]. In addition, Dugesia Tigrina is capable of asexual reproduction facilitating their selection. Selection of individuals for the experiment was carried out with close morphometric parameters. The planaria for this experiment was contained in a plastic polypropylene container. The daily temperature fluctuations in the room ranged from 19 to 22°C, the duration of daylight hours was 25 min, since planariums were mainly contained in the artificially darkened room, and were removed from it only for daily measurements. Because of their sensitivity to the composition of water [12], water was taken from the place of origin (Institute of Theoretical and Experimental Biophysics) of the planarians for their dilution (Pushchino artesian well, Pushchino, Moscow Region, Russia, pH = 6.5, salinity 1.5-2.0 mg/l). During the experiments the temperature was maintained at 26±1°C for 24 h.

The experiment consisted of decapitation of the planaria (Institute of Theoretical and Experimental Biophysics, Russia) using a sterile scalpel. Two groups (control and experimental one) including ten planaria in each were distinguished. For both groups, planarians with a trunk length of about 10 mm were selected. The decapitation was carried out in such a way that the upper part of the head was cut off above the planaria eye line. In the experimental group, immediately after decapitation, the planaria was irradiated with hypersound using the setup proposed in [13]. A silicon wafer illuminated by a white LED illuminator with a color temperature of 6000 K was used as a hypersound source. As shown experimentally in [13], light irradiation of silicon containing on the surface a layer of natural oxide (NO) with a thickness of about 2 nm lead to the process of redistribution of the charge of NO and the appearance of an electric field with a strength of the order of 10⁷ V/cm. In this case, a periodic breakdown of a thin layer of NO occurs, which, as a result of the piezoelectric effect, lead to the excitation of acoustic oscillations with a frequency of about 10¹⁰ Hz. Acoustic vibrations propagate through the thickness of silicon without attenuation [14] due to the waveguide properties of dislocations. Turning to the side opposite to the irradiated, an acoustic wave propagates along the solid – liquid interface. Due to the tight contact of silicon with glass, a thin layer of water was formed between them. The surface of the glass contains Na⁺ ions, since it is part of the glass (11% by weight). The water-glass interface contained water clusters Na⁺ – [H₂O]ₙ [13]. In this work, it has been shown that a chain of Na⁺ – [H₂O]ₙ, clusters conducts hypersound of the indicated frequency better than water in the volume of the Petri dish. Hypersound along the water-glass interface reached the planarium and influenced on them. The experimental design of the irradiation is shown in figure 1. A KDB-1 (Russia) in (111) crystallographic orientation silicon wafer with a thin layer of natural oxide on the surface was used as a
source. The silicon wafer was illuminated with light from a 10 W LED illuminator and a color temperature 6000 K from a distance of 7 cm. So in a glass Petri dish (100 x 15 mm, nunclon™ delta, Nunc) at the glass-water interface contains Na⁺ ions. For this reason, silicon was located on the surface of the glass, and a thin layer of water remained between the glass and silicon. In order to prevent light from entering the planarium during the irradiation of silicon, silicon was placed in a Petri dish with planarians, the planarium with silicon was covered with the glass cover upside down on top, and everything except silicon was covered with black paper on top. The irradiation of silicon was carried out for 100 s. This time was chosen because in previous works [2, 13] it turned out to be optimal from the point of view of the magnitude of changes in the properties of silicon. After irradiation, the experimental and control groups were sent to the thermostat (TS-1/80 SPU, Russia) for 24 h at 26±1°C.

![Figure 1. Experimental scheme for bio long-range effect testing: 1 – LED, 2 – screen, 3 – silicon plate, 4 – Petri dishes, 5 – water, 6 – bio-cell.](image)

In the work, manual counting of microorganisms from the planar habitat for each control group was carried out under an optical microscope Biomed var 3-1 with a Toupcam 5.1 MP – Micromed video lens through TouView 3.7 software.

3. Experiment

During the experiment, a number of conditions coincided, such as: 1) the presence of various microorganisms and cysts in artesian water of the initial content of planaria; 2) an increase in water temperature up to 26°C (for aqueous organism in our area it was a high temperature); 3) the presence of a large amount of nutrients (intercellular substance planarium that has fallen into the water after the decapitation process). As a result of the coincidence of these conditions, the microflora and microfauna of the contents of Petri dishes have grown rapidly. After decapitation, the planar speed decreased to a few cm per minute; the preferred direction of movement was not observed. However, most often they were at rest.

The decrease in the number of planarians in Petri dishes during the time of the experiment created the need for microbiological analysis of water, which contained the Experimental and Control groups, as well as water from the box of the early (Initial) content of the population, hereinafter indicated as “E”, “C” and “I”, respectively.

Group “I” contains: a representative of the order *Amphipoda* (presumably Niphargoides maeoticus;) and dozens of representatives of *Brachionus rubens* (rotifers). Group “E” contains: hundreds of representatives of *Coleps hirtus* (Ciliates of the Prostomatida Squad), dozens of representatives of *Stylophchia* (Ciliates of Stilonychia), dozens of representatives of *Brachionus rubens* (rotifers) and single representatives of *Cyclops sp.* (Cyclops squad). Group “C” contains: hundreds of representatives of *Coleps hirtus* (Ciliates of the Prostomatida Squad), dozens of representatives of *Stylophchia* (Ciliates of Stilonychia), dozens of representatives of *Brachionus rubens* (rotifers), and single representatives of *Cyclops sp.* (Cyclops squad).
Coleps hirtus (Ciliates of the Prostomatida Unit) are of particular interest; they are called the "hyena" of the protozoa world, the basis of their diet is the organic remains of other unicellular organisms. Colepses can also, alone or in groups, attack living, but damaged organisms, in our case decapitated parts of planaria. Coleps uses toxicants, which are organelles containing poison. Coleps injects poison into his victim and waits until the prey is paralyzed, usually about 5-10 min. If the “production” exceeds the size of the Colepses, then they will cling to it until the toxicants become effective and paralyze the “production”. We saw this mechanism in action after 24 h from the moment of decapitation, when in the places where the planaria was cut using a microscope, several rows of ciliates clinging to the scar of the planaria could be seen. After a week of daily observation of the Petri dish with the control group, not a single planarium gradually remained in it, and in the experimental group more than half (60%) of the worms remained (table 1). In the experimental group, the behavior of the planarians has undergone changes. So, if before the experiment the planarians preferred to dwell on the bottom of the Petri dish and had a high speed of movement, then after a week of the experiment most of them moved to the walls of the Petri dish or to the water-air interface; most of the planarians began to lead a passive lifestyle, moving only with point mechanical action.

Table 1. The content of microorganisms in the habitat of planaria before (group “I”) and after the experiment.

| The content of the Petri dishes | Experimental group | Control group | Initial group |
|--------------------------------|--------------------|---------------|--------------|
| Amphipoda (amphipods)         | -                  | -             | ~10^5        |
| Brachionus rubens (rotifers)   | 10^1               | ~10^1         | ~10^9        |
| Coleps hirtus (Ciliates Prostomatida Squad) | 10^2               | 10^2          | ~10^9        |
| Stylonychia (Ciliates Stilonychia) | ~10^1             | ~10^1         | -            |
| Cyclops sp. (Cyclops squad)    | ~10^9              | ~10^9         | -            |

4. Simulation

4.1. Computer simulation method

Since the waveguide model of Na^+–[H_2O]_n clusters was tested in this work, molecular dynamics modeling was performed using the LAMMPS code [15]. To build the model, the atomsk code [16], Ovito [17] was used, the latter was also used to visualize the simulation results.

A model of the waveguide chain chain of Na^+–[H_2O]_n clusters was created using the interaction potentials ReaxFF [18]. The geometry of the model was a parallelepiped with dimensions 3.3 nm × 3.3 nm × 132 nm, consisting of water molecules placed in the periodic Born–Karman boundary conditions in all three dimensions. In the first case, the parallelepiped contained Na^+ ions located with a period of 3.3 nm; in the second case, only water molecules. The system was brought into its initial state from a solid phase of ice with hexagonal packing [19] by melting to a liquid phase with a temperature of 300 K. After preparation, the propagation of a hypersonic acoustic wave was calculated using the deviation of the center of mass of the layer (1.8 nm thick) of water molecules from the equilibrium position with respect to the law 0.01nm·sin(2π/10^13·Hz·t). The reported study was supported by the Supercomputing Center of Lomonosov Moscow State University (Russia) [20].

4.2. Computer simulation results

The simulation results of the propagation of a hypersonic acoustic wave were used to plot the amplitude of the deviation of the layers of water molecules 1.8 nm thick from the equilibrium position. The graph of the dependence of the deviation amplitude on the distance to the source (figure 2) shows that, starting from 10 nm, the wave amplitude in the system containing Na^+ ions reaches a plateau and is 5 times
higher than in a system without Na$^+$ ions. This result indicates that the chain of clusters created by Na$^+$ ions contributes to better wave propagation and less attenuation.

![Figure 2. Dependence of cluster motion magnitude vs distance from the hypersonic source.](image)

In this work, using the molecular dynamics method, it is shown that the propagation of a hypersonic wave along a water-glass interface containing sodium ions occurs further than in an aqueous medium. The simulation results confirm the hypothesis of an abnormally distant distribution of hypersonic effects through a chain of clusters. And the experimental data indicate the result of the impact of hyperson on the regeneration and survival of planarians after decapitation. The agreement of the results of these experiments is that both experiments confirm the mechanism of the long-range effect. Hypersonic exposure possibly inhibits the development of predatory microorganisms, which contributes to better survival of planaria. In addition, the effect of hyperson can affect the regeneration process in planarians as was shown for the magnetic field in [8].

**Conclusion**

According to the results obtained, a long-range effect takes place in the silicon-water-glass-biological object (planaria) system. According to the proposed mathematical model, the mechanism of transmission of the effect of a hypersonic wave of $10^{10}$ Hz is based on the excitation of a cluster chain of Na$^-$ – [H$_2$O]$_n$, which serves as a waveguide. Two possible mechanisms of the effect of a hypersonic wave on planaria through microorganisms of predators living in the aquatic environment along with planaria and through a change in the processes of regeneration of planaria have been identified. Both of these mechanisms are subject to further experimental quantitative verification, as well as the creation of mathematical models.

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