

Research Article

Evaluation of the Influence of Super-Low-Intensity Microwave Radiations on Mesenchymal Stem Cells

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Abstract

This paper is devoted to the analysis of advanced scientific research on the role of super-low-intensity microwave effects on biological objects in form of stem cells. The scientific work has been performed by a translational team of researchers, including doctors, engineers, radio physicists, and programmers. Synchropulsar AQUA was used as a source of super-low intensity <10 μ W of electromagnetic microwave radiation in the 1 GHz band. During the study, it was found that the number of cells is reduced in the experimental groups compared with the control. In the control it was calculated 3.1 $\times 10^6$ cells/mL, in the low frequency biomodulation mode it was found 2.5 $\times 10^6$ cells/mL; in the continuous generation mode it was revealed 2.1 $\times 10^6$ cells/mL. Assessment of the fluorescence intensity showed that the activity of mitochondria is higher in the experimental group under irradiation with mode modulation by biotropic parameters - 12.40 conventional units (cu). When using regime continuous generation, the activity of mitochondria was 9.33 cu. In the control group, mitochondrial activity was 10.74 cu.

Keywords: Microwave Radiation; Centimeter Waves; Super-Low Intensity; Regime Low Frequency Biomodulation; Regime Continuous Modulation; Fluorescent Method; Mitochondrial Activity

Introduction

Currently, tissue engineering is trying to use scientific knowledge and advances in the development of new medicines and methods of treatment in various areas of medicine to accelerate the regeneration or replacement of tissues, such as bones, cartilage, tendons and ligaments. Today, the standard tissue engineering approach for solving the problems of tissue

repair and regeneration supposes the joint use of composite materials in the form of three-dimensional matrices, growth factors, and multipotent stem cells. Stem cells are non-specialized cells with the ability to differentiate (become another type of cell) and self-renewal (reproduce themselves without differentiation). The definition of stem cells is based on their innate properties such as self-renewal and differentiation [1,2]. In the scientific literature various ways of differentiation of stem cells are considered in detail [3,4]. The process of cell differentiation is realized under the influence of internal and various external factors. They include neurohumoral regulation and external factors in the form of rhythm of activity, light, and temperature, and electromagnetic radiation [5]. It should be emphasized that stem cells are located in the environment of electromagnetic radiation of the information level emanating from tissue cells. It is necessary to note that stem cells exist also in the environment of chronobiological rhythms, such as circadian rhythms, cycles of hemodynamic, respiration, microcirculation, and periods of protein elongation on ribosomes. All chronobiological problems are reflected in the history of the development of technical means for informational impact on various biological objects, as well as human tissues and organs. In 1995 for the first time was patented the biotechnical system of the millimeter therapy includes an avalanche transit-time diode and operates in the cyclical

mode of controlling by means of changing the duty ratio of signals. The control was implemented intact with the beats of the pulse and breathing of the patient [6].

In the 90 years of the 20th century were obtained principally new interaction mechanisms of biological objects with super low doses of biologically active substances [7]. Besides that, in the last years of the XXI century in the study of the impacts on biological objects by means of short-term and super weak intensity EHF radiation was detected similar result [8]. Also is noted negative effect of EHF radiation on the growth of microbial flora and fungi (black mold) [9,10]. It should be especially emphasized that the authors of a joint Russian-Italian scientific study revealed the facts of the influence of microwaves on the human genetic apparatus [10]. Along with this, a resonant influence on the state of the genome structure of *E. coli* cells was revealed [11]. In the scientific literature of millimeter therapy debated question about the effectiveness of short-term and super weak radiation EHF at an intensity of 10-16-10-20 Wt/cm² Gunn diode. For 1995 year the known also that water becomes radio transparent for frequencies of millimeter range 64.6 и 65.7 GHz of super-weak intensity $\leq 10 \mu\text{W}/\text{sm}^2$. For this reason, such radiation can penetrate in aquatic environments with very low losses energy [12,13]. Summarizing the above presented literature review, it should be concluded about that super-low-intensity electromagnetic radiation has a significant effect on various biological objects. Therefore, the study of the effect superweak microwave on stem cells relates to an urgent task. The purpose of the study was formulated as establishing the fact of the influence of a super-weak electromagnetic field with a frequency of 1 GHz and a power of less than 10 μW on mesenchymal stem cells: in the mode of continuous generation and in the mode of low-frequency modulation by human biotropic parameters.

Research objectives include:

1. Creation of the structure biocontrolled super-low intensity centimeter wave generator
2. Development of low frequency pattern modulation of super-low-intensity microwave radiation by means of model signals including heart rate, signals of metronomized breathing, rhythms of microcirculation and protein elongation rhythms on ribosomes
3. Formation a continuous generation mode using microcontroller and timer unit
4. Creation of the structure of a cyclic algorithm for controlling microwave radiation with a given signal duration
5. Investigate the effect of microwave irradiation in a super-low intensive mode on proliferation and activity mitochondria stem cells
6. To compare the effect on stem cells of super low-intensity microwave radiation with a frequency of 1 GHz in the low-frequency biomodulation mode and in the continuous generation mode

Materials and Methods

The study was performed using human Mesenchymal Stem Cells (MSC) extracted from adipose tissue. The cell culture was purchased from Biolot LLC, Russia (Fig. 1-3).

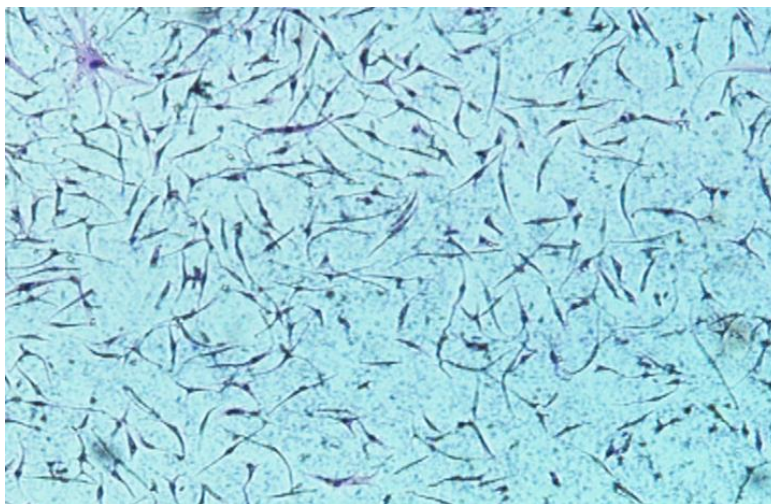


Figure 1: Human mesenchymal stem cells. Increase x4.

Synchropulsar AQUA was used as a source of super-low intensity $<10 \mu\text{W}$ of electromagnetic microwave radiation in the 1 GHz band.



Figure 2: Operating modes of the Synchropulsar Aqua device. Left: Power toggle is in pose turn on; Right: Switch of dual mode is in the position continuous generation.

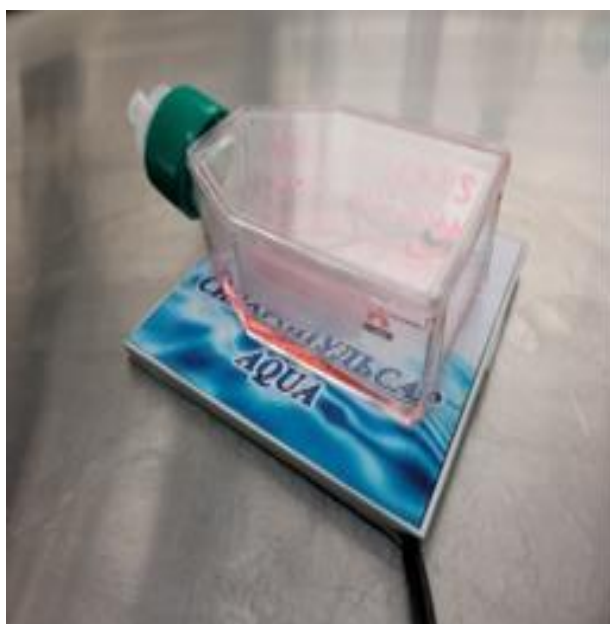


Figure 3: Left: Flacon with culture of the cell is located on the antenna; Right: Flacon packed in foil.

The duration of the experiment was 9 days. Once a day the flacons of the experimental group were installed for 15 minutes on a loop antenna. Mesenchymal Stem Cells (MSC) were cultured in flasks with complete nutrient medium DMEM/F12 with 10% fetal calf serum (PanEco, Russia). After irradiation, culture flasks with MSC were placed into CO₂ incubator and cultured at 37°C, 5% CO₂, 100% humidity. After completion of the experiment, cell proliferation was assessed by counting the number of cells using a Scepter 2.0 cell counter with 40 μm tips (Millipore, Merck/Sigma-Aldrich, USA). Mitochondrial activity was studied using a Nikon Ti-S fluorescent microscope (Nikon (Japan) with specialized software Nikon EZ-C1 FreeViewer (Nikon, Japan) using MitoTracker™ Red CMXRos fluorescent dye (ThermoFisher MitoTracker Red CMXRos is a red fluorescent dye that stains mitochondria in living cells. This dye is fluorescent only when oxidized in the cell, an alkylating chloromethyl group is attached to this dye. By virtue of their membrane potential, functional mitochondria absorb the dye (Fig. 4).

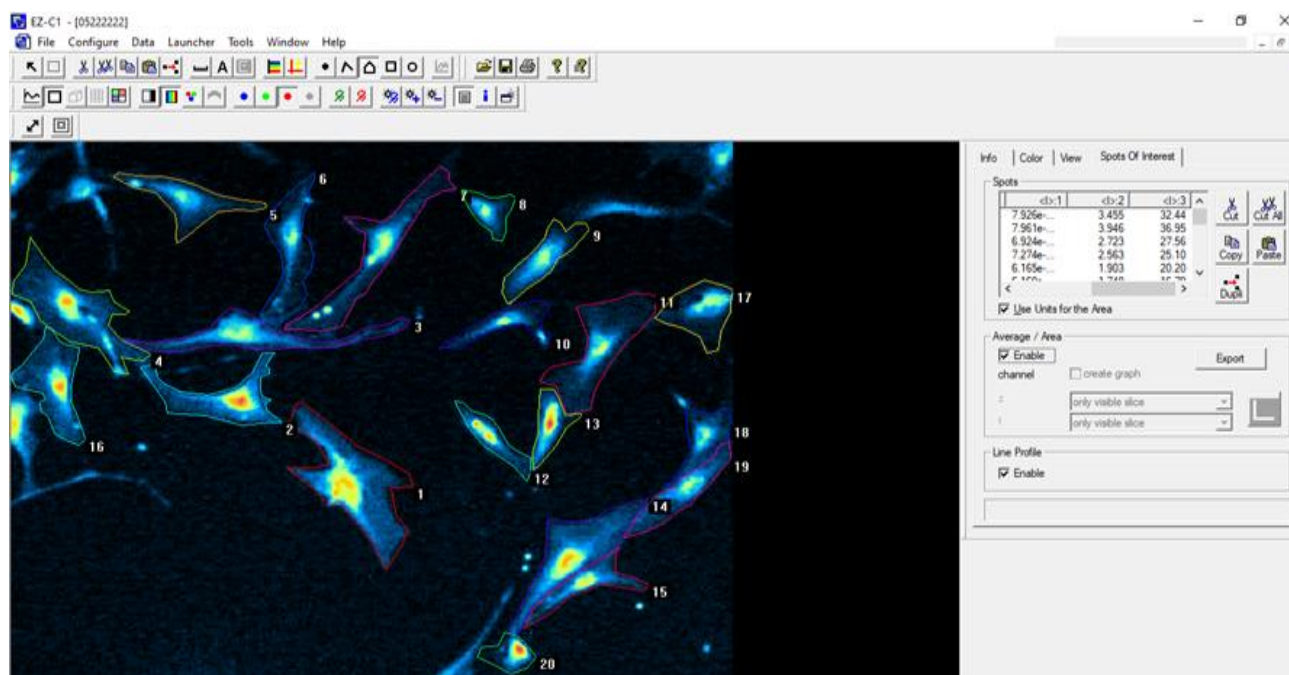


Figure 4: Interface of the Nikon EZ-C1 FreeViewer program (Nikon, Japan), with outlined MSC.

Statistical processing of the results was carried out using the statistical software package STATISTICA 6.0 (StatSoft Inc., USA). Data were considered significant at $p < 0.05$. The nonparametric Wilcoxon test was used.

Research Results

During the study, it was found that the number of cells is reduced in the experimental groups compared with the control. In the control it was calculated 3.1×10^6 cells/mL, in the low frequency biomodulation mode it was found 2.5×10^6 cells/mL; in the continuous generation mode it was revealed 2.1×10^6 cells/mL (Fig. 5,6).

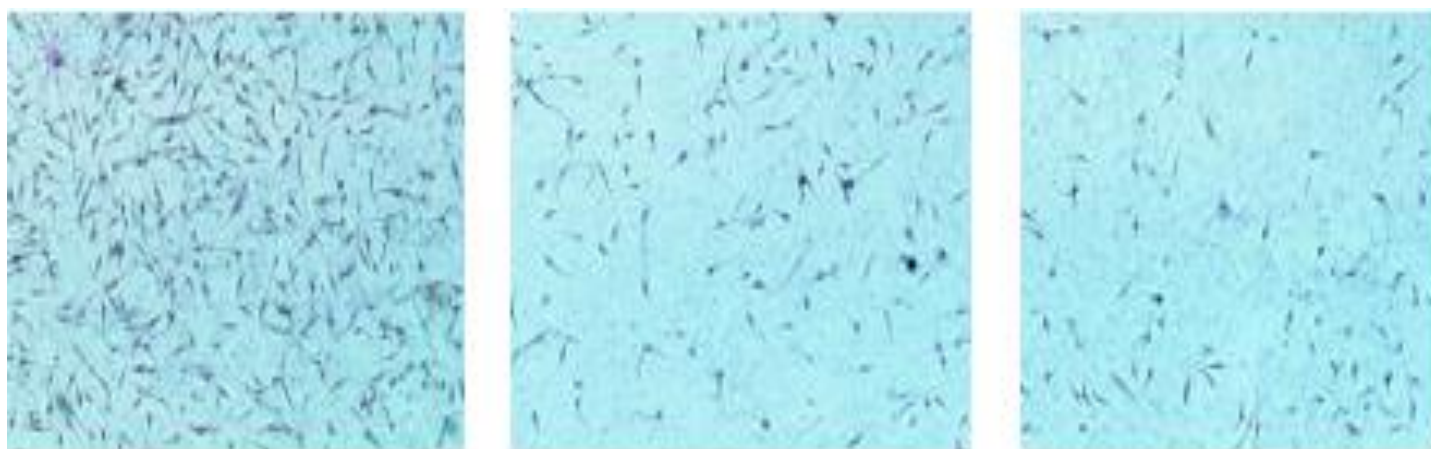


Figure 5: Influence of various regimes of super-low-intensity microwave radiation on the processes of stem cell proliferation. Left: control - 3.1×10^6 cells/mL; Middle: low-frequency biomodulation mode - 2.5×10^6 cells/mL. On the right: continuous generation mode - 2.1×10^6 cells/mL.

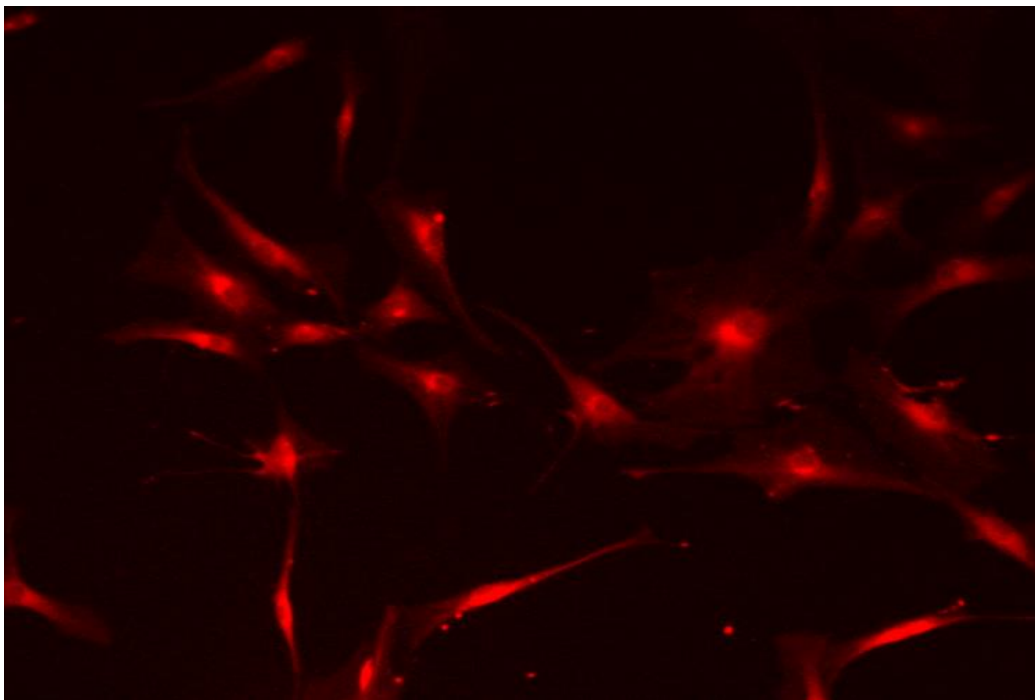


Figure 6: Fluorescence of MSC in the control group.

Assessment of the fluorescence intensity showed that the activity of mitochondria in the control was 10.74 conventional units (cu). When using the low-frequency modulation mode, the fluorescence increased to 12.40 conventional units and when impact by means of continuous generation mode, the fluorescence intensity has decreased to 9.33 conventional units. Thus, a comparative fluorescence analysis of mitochondria shows that the use of low frequency biomodulation leads to a significant increase in mitochondria activity (Fig. 7,8).

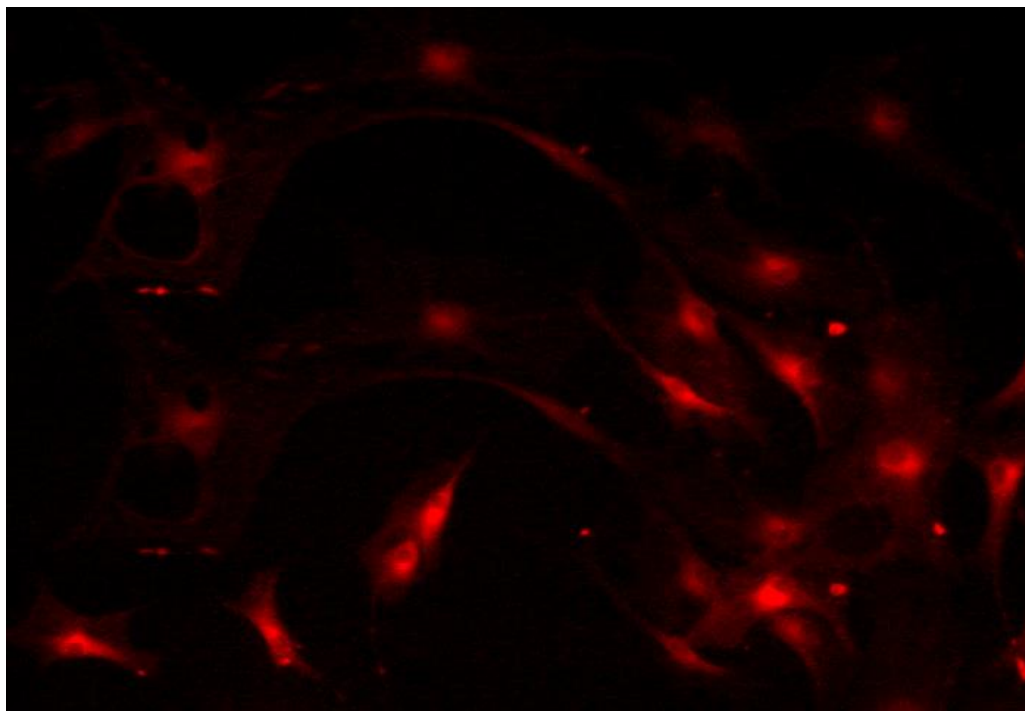


Figure 7: Fluorescence of MSC from the experimental group in continuous generation mode.

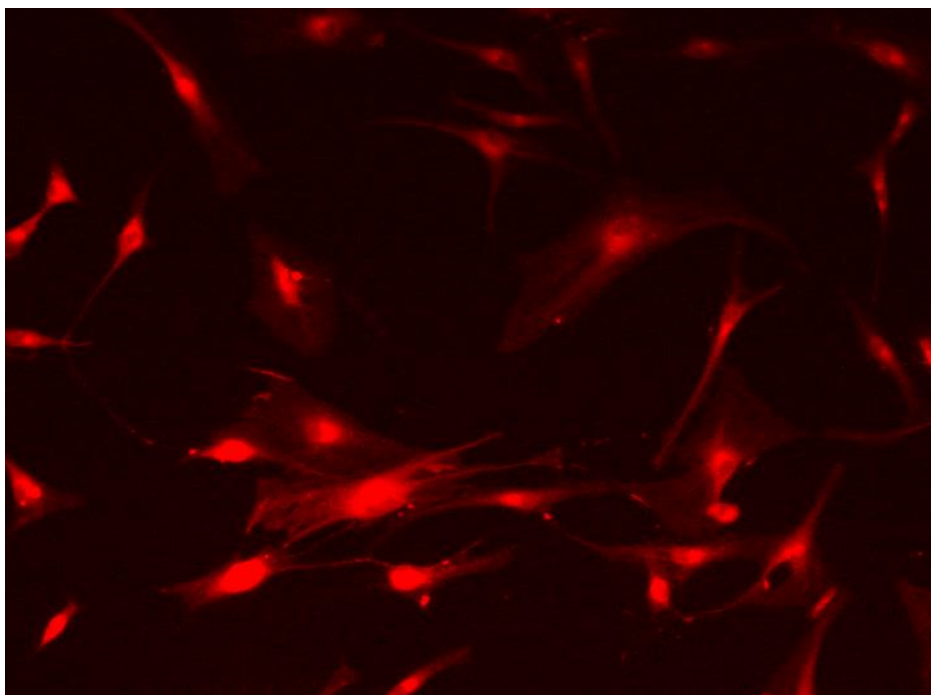


Figure 8: Fluorescence of MSC from the experimental group in low frequency biomodulation mode.

Discussion of the Results of The Study

Let us consider what are the mechanisms of the observed changes in mesenchymal stem cells that occur under the influence of super-low-intensity microwave radiation? At the beginning of the 21st century the models of the living state of the cell were being studied many modern eminent biologists [14,15]. However, it was reported that these scientists did not take into account the problems of cell bioenergetics. That is why these models have not become accepted theories.

All biochemical and physiological processes are realized in the aquatic environment. Only in 2009 a new concept of the molecular mechanism of energy transfer, as well as the perception of super weak impacts by living systems, was proposed by scientists from St. Petersburg [16]. Later all the provisions of this concept were well-grounded.

Further, the views of the cited authors on the problem under consideration are presented. The concept was based on the methods of nonlinear mathematical physics in describing the movement of energy along molecular chains and on quantum mechanical concepts of the formation of signals in anisotropic media. The idea of a molecular unit as a single structural module - an integral part of a biological system, which displays the collective properties of the oneness of molecules, an energy-tense and connected aqueous medium and physical fields in the processes of perception and energy transfer, has been put forward and substantiated. It is shown that intermolecular energy transfer and amplification of superweak effects are elements of a single energy process in a living system, while the physical basis of both processes is the oneness of the molecular and water-field environment of a molecular module. Thus, in the latter case a molecular module defined as a temporary formation of two or more biopolymers that is closest to each other for some time, but still separated by an aqueous medium.

And now we will consider the "life process" of a molecular module. As became known, a molecular module includes three mandatory participants: biopolymers (at least 2 or more), an aqueous medium (a fractal "fur coat"), and an electromagnetic field in the form of soliton waves. All of them interact in a non-linear but regular way, as a result of which the molecular module and displays the properties of a "living system". The molecular modules that make up cells habitual to us are not independent systems in the sense that they "do not feed themselves" but need continuous energy supply from the external environment. First of all, under the action of energy given off by solitons in the "discharge" mode, water surrounding biopolymers in living systems crystallizes with the formation of fractal crystals fixed on hydrophilic (having affinity for water) regions of biopolymers. At the same time, water fractals, behaving like crystals - antennas, simply re-radiate the rest of the energy to the rest of the polymers of the molecular module. Each of the biopolymers that have received chemical energy from ATP (and all of them have "fur coats"

of fractal water crystals around them) not only uses it itself, but also exchanges with its neighbors, transferring excess energy to other biopolymers of the molecular module. Quite so, the communication between biomolecules is supported, and their conformations become mutually concordant.

In fact, we have just considered a two-structure dynamic fractal-cluster model of the supra molecular organization of the liquid phase of water. The model includes six-molecular molecules $(\text{H}_2\text{O})_6$ - hexagons and H_2O monomolecules - triads. In the process of metabolism, two-way migration of triads into the cavities of hexagons can occur with the formation of complexes $[(\text{H}_2\text{O})_6 + \text{H}_2\text{O}]$, or so-called clathrates. Dynamic integration of clathrates into clusters of the n th order leads to the appearance of fractal associates $6n [(\text{H}_2\text{O})_6 + \text{H}_2\text{O}]$. The resulting structure resemblant "matryoshka" doll, in which, under external action of microwave super-low- intensity radiation occurs resonant excitation of an associate, as a whole and, as well as molecules independent of it, namely: triads, hexagons, clathrates and clusters. After the termination of the external impact, the re-radiation of the cluster structures into the environment immediately begins. This re-emission is maintained indefinitely after the termination of the external influence.

Associated water, in addition to all of the above, provides a high degree of hydration of protein and protein-lipid structures in cells, and also allows it to stimulate effective intracellular electron transfer and transport of intracellular metabolites, as well as to have a stabilizing regulatory effect on the cell cycle.

Consequently, the primary target for impact by means of super weak microwave field is structural formations of water: hexagons, clathrates, clusters and associates, which, changing, lead to the transformation of membrane properties and, as a result, to a change in the functional activity of stem cells.

When using the mode of low-frequency modulation of microwave radiation based on biorhythms of the cardiac cycle, resonant respiration, and rhythms of protein elongation on ribosomes, we obtained signs of an increase in the intensity of mitochondrial luminescence.

This, apparently, is associated with the processes of synchronization of oxidative and energy metabolism and, in turn, indicates an increase in the activity of mitochondria. A decrease in the number of MSC in the experimental group when using the continuous generation mode may indicate the start of the process of MSC differentiation.

Conclusion

1. A special device "Synchropulsar Aqua" was developed to study the effect of super-low intensity electromagnetic radiation on human mesenchymal stem cells
2. The device operated at a frequency of 1 GHz and a radiation power of less than 10 μW and included two modes: continuous generation and low-frequency modulation using biotropic parameters of pulse, breathing metronomized, cycle's microcirculation and rhythms of elongation protein on ribosomes
3. A statistically significant decrease in the number of mesenchymal stem cells was established in both modes of impact. However, when using the mode of low-frequency modulation by biotropic parameters of super-low-intensity microwave radiation, a significantly smaller decrease in the number of mesenchymal stem cells was noted
4. An increase of the fluorescence of mesenchymal stem cells was revealed when impacting factors were microwaves with the low-frequency modulation rhythms namely: the pulse rate, metronomized breathing, microcirculation rhythms and protein elongation on ribosomes. The obtained facts testify about the activation of mitochondria of mesenchymal stem cells
5. It is especially important to note that a decrease in the number of MSC in the experimental group when using the continuous generation mode perhaps indicates the start of the process of MSC differentiation

Conflict of Interest

The authors have no conflict of interest to declare.

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