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Effects of infrared and ultraviolet radiation on the viability of cells immobilized in porous TiNi-based alloy scaffold

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ABSTRACT

Cell responses to electromagnetic radiation are due to many factors including the cellular microenvironment. The aim of the present study was to explore the effects of ultraviolet (UV) and infrared (IR) irradiation of low intensity on cultured cells derived from different biological tissues (spleen, bone marrow, and Ehrlich's adenocarcinoma), which were immobilized in a porous TiNi-based alloy scaffold. Accordingly, the following objectives were set: i) to evaluate the impact of low-intensity radiation on cell suspensions, and ii) to carry out a comparative analysis of the viability of cells immobilized in porous TiNi-based alloy and IR- and UV-irradiated. The data show that the extracellular environment of bone marrow, tumor and spleen cell populations affects their viability and proliferative potency in porous TiNi-based scaffolds. IR- and UV irradiation of cell cultures immobilized in the scaffold affects the cell viability in populations of bone marrow, tumor, and spleen cells. In case of IR irradiation, cell viability was significantly improved, at the same time UV irradiation suppressed cell proliferation activity. The effect of IR irradiation can be used to resuscitate the cell area. The effect of UV irradiation can be used to destroy residual tumor lesions or other pathological cell populations. Effects of low-intensity infrared (IR) and ultraviolet (UV) radiation on the number of viable cells were evaluated against the control group in which cells were exposed to natural daylight. The results showed that IR irradiation led to a 4.6-, 2.5-, and 1.3-fold increase in viable Ehrlich tumor, bone marrow, and spleen cells, respectively, while UV exposure led to a 3.9-, 1.5-, and 1.2-fold increase, respectively. Copyright © 2015 VBRI Press.

Keywords: Cell cultures; cell tissue engineering; porous TiNi scaffold; IR and UV radiation.

Introduction

The introduction of long-term cell culture techniques, including the ones for progenitor cells of specialized tissues, has paved the way for the development of new technologies for cell and tissue replacement therapy and bioartificial organ engineering in experimental biology and medicine [**1, 2**]. One of the most promising approaches to use cultured cells is the possibility to transplant them into the damaged areas. The success of the treatment depends on the number of cells reaching the damaged area, the capacity of cultured cells to adhere to the target tissue, and their ability to maintain an active functional state. Providing optimal healing conditions at the recipient bed is of high importance for the development of transplantation technology for *in vitro* grown cells. Long-term preservation of the functional activity of *in vivo* implanted cells remains a serious problem. Simple introduction of a progenitor cell suspension has been found to be ineffective. Therefore, finding an adequate carrier for transplantation of cells into a recipient organism is critical [**3-7**].

The interdisciplinary approach used in cell and tissue engineering primarily aims at the creation of new composite materials for the recovery of lost functions of individual tissues or whole organs. The main principles of this approach are to develop and implant different biomaterial carriers together with donor cells and/or bioactive substances into the damaged organ or tissue [**8-9**]. Materials to be used for tissue engineering are required to have specific characteristics. Until the new host tissue is completely restored at the implantation site, the material used for manufacturing the implanted construct must support growth of cells and their organization into the tissue. Besides, the implant must allow unrestricted diversion of cellular metabolic products [**8**].

Matrices (scaffolds) must be multifunctional, in particular, possessing elasticity and the necessary mechanical strength, biocompatible at the cell and protein levels, able to support cell attachment and stimulate cell proliferation and differentiation, and able to sustain sterilization without changing their medical and technical

properties. To realize their potential, the cultured cells have to remain fixed to the carrier for a certain time for histogenetic properties of these cells organized in complex three-dimensional structures to be manifested [**8-10**].

In recent years, porous permeable TiNi-based alloy scaffolds have been actively used in the development of biocompatible materials for tissue engineering and transplantation. It is promising to use these biomatrices for regeneration of gland and liver tissues damaged by tumors and other etiologies, manufacturing bioimplants of blood vessels and hollow organs, and closing defects both soft and bone tissues [**11-13**]. Methods for selecting the required number of viable cells do not always provide desired results. In addition, the quality of tissue taken from a patient varies and depends on many factors, including the extent of the disease, the patient's age, the punctate amount, etc. Thus, one of the critical problems of cell transplantation is to obtain the required number of viable cells for transplantation. Different authors offer various solutions to this problem, e.g., improved methods of cell isolation, the use of larger amounts of biological material, and the use of diverse biostimulants, including both chemical and physical factors, to promote cell growth and proliferation [**14-16**].

The human body, as an open thermodynamic system, absorbs and releases energy, including the energy of electromagnetic radiation. In addition, body tissues respond to all energy changes at the cellular level. The impact of radiation on cellular biosystems is determined by a number of factors, such as the wavelength, radiation energy density, and pulse duration, which may have significant effects on the cell populations of the body. In this respect, the intercellular space and the environment both can play a significant role. The most complex and, at the same time, important physical factor affecting cellular processes is electromagnetic radiation (EMR). The cell's biofield is constantly changing under the influence of many different types of EMR, existing both between cells and tissues and with participation of the biosphere space. Slight variations in the parameters of radiation can cause a change in the cell response and even lead to cell death. Therefore, if the experiment is not well designed and the initial conditions of the study are set incorrectly this can lead to unreliable results [**16, 17**].

Electromagnetic waves modify the state of the lipid bilayer cell membrane, which may increase or reduce water absorption by cell cytoplasm, as well as change membrane polarization and thereby the signal system of the cell. With an increase in the radiation intensity, some effects become more profound such as improved blood flow in the capillaries [**17**]. When tissues are exposed to infrared (IR) radiation, the energy is absorbed by water, oxygen, and enzymes molecules, cell membranes, and other structures. The heat released as a result of exposure to radiation increases the vibrational energy of the molecules and leads to changes in the entire thermodynamic system. IR radiation enhances cell biological activity, accelerates the blood flow, increases glandular activity, relieves muscle spasms, reduces pain, etc. [**17**]. Ultraviolet (UV) radiation changes the properties of biopolymers, primarily proteins and nucleic acids. One of the examples is protein denaturation caused by ultraviolet radiation. Biopolymer molecules contain ring groups which absorb short-wavelength (~280 nm) radiation and actively resonate. This absorbed energy can be passed down to a chain of atoms within a molecule without any significant loss until it reaches and destroys weak bonds between atoms. The process called photolysis generates molecular fragments, free radicals and ions, which have a strong effect on cellular structures. Interaction between UV radiation and chemicals, including organic substances, often causes ionization, which is very similar to the photoelectric effect and changes biochemical reactions in the cells [**16, 17**]. Thus, cell responses to electromagnetic radiation are influenced by many factors, including the cellular microenvironment. The aim of the present work was to study the effects of UV (370 nm) and IR (860 nm) electromagnetic waves of low intensity on cultured cells derived from different biological tissues (spleen, bone marrow, and Ehrlich's adenocarcinoma), which were immobilized in the porous TiNi-based alloy scaffold (patent RF #2438699 "A method of vaccine production for the treatment of Erlich's adenocarcinoma in an experiment", published January 10, 2012) [**18**].

Accordingly, the following objectives were set: i) To evaluate the impact of low-intensity radiation on cell suspensions, and ii) To carry out a comparative analysis of the viability of cells immobilized in porous-permeable TiNi-based alloy after exposure to IR and UV radiation.

Experimental

Scaffolds

For the experiments, porous permeable scaffolds of TiNi-based alloy developed in the Research Institute of Medical Materials (Tomsk) were used. The porous scaffolds having permeable porosity of 65-75% (**Fig. 1**) were produced by the method of self-propagating high-temperature synthesis (SHS). Scaffold samples were cut from a porous ingot of TN-10 brand alloy by the method of electrical discharge machining. The surface topography of the samples and the structure of the pore space were studied under a scanning electron microscope (Quanta 200 3D).

Fig. 1. Porous permeable TiNi-based alloy scaffolds (a) and single item (2.5x2.5x4 mm) (b) used in the study.

Animals

C57BL/6 mice (20-24 g, age 12-18 weeks, males) were used for cell isolation. All animal procedures were carefully carried out with strict adherence to European Convention

for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986) and with the European Communities Council Directive 86/609/EEC. The study protocol was approved by the Bioethical Committee of Siberian State Medical University. All manipulations were performed under general anesthesia. All the animals were sacrificed with pentobarbital sodium overdose (Nembutal, intraperitoneal injection 200 mg/kg m.c.).

Cells

Ehrlich's carcinoma cells (ascites variant, inoculation dose 5×106 cells) were isolated from the ascitic fluid of a C57BL/6 mouse by centrifugation. Then, the cells were resuspended in a complete culture medium consisting of RPMI-1940 medium (Paneco LLC, Moscow) with 10% fetal calf serum, 250 mg/L glutamine and 40 μg/mL gentamicin (Paneco LLC, Moscow). C57BL/6 mouse bone marrow and spleen cells were isolated in the same medium, following published procedures [**19**].

Treatment

The cells were seeded in 96-well plates at a density of 2×105 cells per well. Sterile samples of porous TiNi-based alloy (2.5×2.5×4 mm) were added into the wells to ~1/10 of the culture volume. A device with integrated LEDs emitting light at predetermined wavelengths was placed on the top. Each LED irradiated three cell suspension wells (n=3). Effects of irradiation on cells samples were studied with the use of LED types L-53SF6C (IR radiation) and LLT-UVLED11 (UV radiation). Cells were irradiated for 4 hours at a distance of 1 cm from the liquid and a radiation power of 4-6 mW/cm2, followed by a 20-hr adaptation period in a CO_2 incubator at 37 °C, 100% humidity in the dark. At the end of cultivation, cells were detached by incubating with 0.25% trypsin-EDTA for 30 min. Then, the plates were centrifuged and cells resuspended. Cell viability was evaluated by the trypan blue exclusion method using 0.4% trypan blue. The percentage of trypan blue-negative cells was calculated relative to the total number of cells, and the data were analyzed statistically.

Comparison groups

The following experimental and control groups were compared: (a) Control: a cell suspension exposed to daylight artificial radiation; (b) Scaffold: a cell suspension immobilized in the porous TiNi-based scaffold and exposed to daylight artificial radiation; (c) IR: a cell suspension exposed to IR radiation; (d) UV: a cell suspension exposed to UV radiation; (e) IR+Scaffold: a cell suspension immobilized in the porous TiNi-based scaffold and exposed to IR radiation; (f) UV+Scaffold: a cell suspension immobilized in the porous TiNi-based scaffold and exposed to UV radiation. The exposure times were the same for the control and experimental cultures.

Statistical analysis of the data

It was performed by the standard methods using the Statistika-6 statistical software package. Since the study included only samples, the law distribution of the numeric parameters differed from the normal distribution, according to the Kolmogorov-Smirnov test. The statistical significance of the differences between the parameters studied was tested by the non-parametric Mann–Whitney U-test, with pair wise comparison of independent sets of parameters.

Results and discussion

Previous studies of the interactions between multipotent mesenchymal cells of the bone and the pore wall surface of porous TiNi-based alloy have shown that the material is biocompatible and has good adhesive properties for this type of cells [**14**]. This was confirmed by an increased number of adherent mesenchymal cells on the inner surface of the porous TiNi-based alloy (**Fig. 2a**). The tissue derived from these cells filled more than 90% of the scaffold pores after 28 days of incubation *in vitro* (**Fig. 2b**).

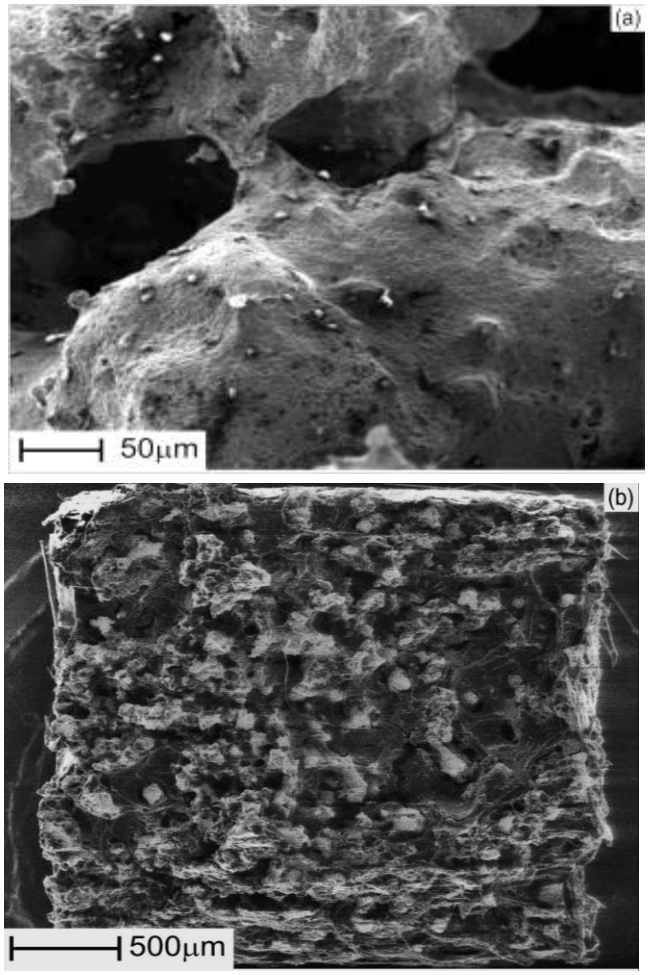

Fig. 2. (a) SEM images showing mesenchymal cells seeded on porous TiNi-based alloy scaffold in a day post-seeding and (b) at day 28 post-implantation, scaffold entirely filled with cells and extracellular matrix.

In our study on the effect of IR and UV radiation on Ehrlich's tumor, spleen and bone marrow cells of C57BL/6 mice, significant effects were observed in the case of cell irradiation for more than 4 hours (**Fig. 3**).

Exposure of all tested cell populations to IR radiation of low intensity resulted in a significant increase in the number of viable cells compared to the non-irradiated

control. In the case of UV radiation, a significant decrease in the viability of cell populations was observed versus the control. In the human body, the cellular microenvironment and cell location within the body play main roles in the protection against various types of radiation. In this study, we investigated the effects of IR and UV radiation on cell suspensions immobilized in porous TiNi-based alloy.

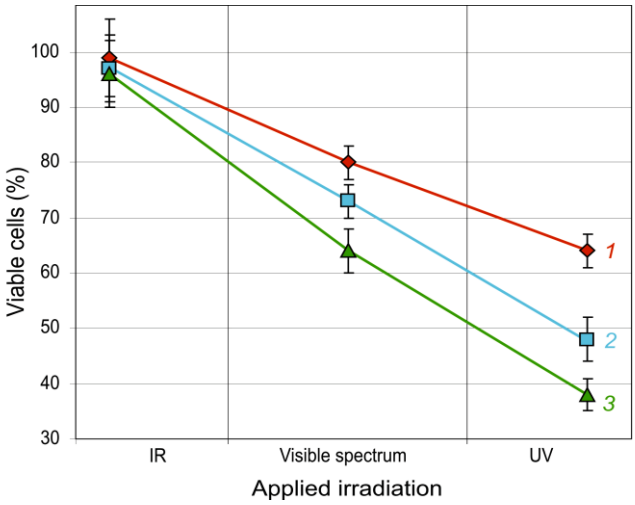

Fig. 3. Vitaity of target cells after they have been subjected to four-hour IR- and UV-irradiation in vitro: 1 - Ehrlich tumor; 2 – spleen; 3 - bone marrow.

We observed significant changes in the viability of cell cultures after their irradiation in the culture medium containing porous TiNi-based alloy. In the experiment, we noticed multiplication of the number of viable cells in the bone marrow and tumor cell cultures and a slight increase of the number of viable cells in the spleen cell cultures for both IR and UV wavelength ranges (**Fig. 4**).

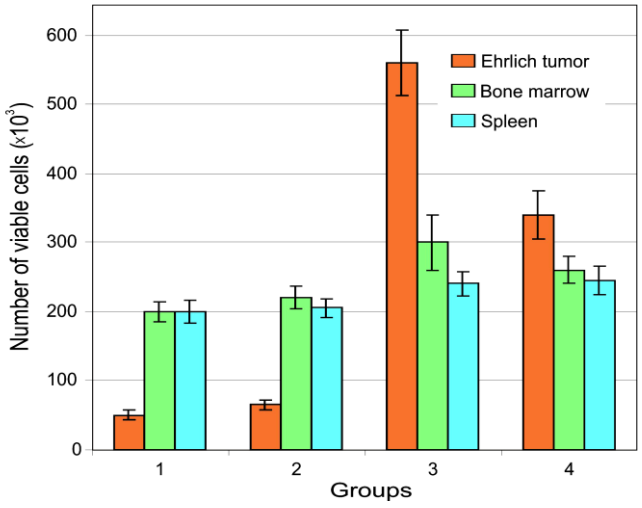

Fig. 4. Number of viable cells (Ehrlich tumor, bone marrow, and spleen) depending on applied effect and conditions: 1-culture medium; 2-porous TiNi-based alloy scaffold without irradiation; 3-TiNi-based alloy scaffold IR-irradiated; 4 - TiNi-based alloy scaffold UV-irradiated.

This cell response to IR and UV radiation is associated with the transformation of the radiation energy in these parts of the spectrum into the heat energy of porous TiNi-based scaffolds. The heat energy is smoothed in the aqueous environment surrounding the scaffold, which manifests itself as a soft temperature gradient modulating cell proliferation. In other words, IR and UV radiation affecting the cellular layer of the scaffold, heats pore walls and the matrix base. The liquid environment prevents from heating to the critical damage temperatures for cells (43-45 °C) due to the permeable structure of the scaffold, which has been shown to have a beneficial effect on the proliferation processes of rapidly dividing stem and tumor cells [**12**]. In addition, the porous scaffold protects cells from direct exposure to UV rays and therefore from their damaging effects.

The data show that the extracellular environment of bone marrow, tumor and spleen cell populations affects their viability and proliferative potency in porous TiNi-based scaffolds. Irradiation of cell cultures immobilized in the scaffold with low-intensity IR and UV irradiation affects the cell viability in populations of bone marrow, tumor, and spleen cells. In the case of IR irradiation, cell viability was significantly improved. In the case of UV irradiation, cell proliferation activity was suppressed.

Conclusion

The effect of irradiation in the IR spectrum can be used for resuscitation of cell populations after their isolation from tissue structures to bring small populations to the desired size. The effect of irradiation in the UV spectrum can be used to destroy residual tumor lesions or other pathological cell populations. Currently, the results of this study are used to develop a scaffold for cell cultures, which would have a small generator of electromagnetic radiation with set parameters of exposure placed in the matrix of the porous structure.

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