

Conference paper

The Effect of Infrared and Ultraviolet Radiation in the Development of the Cells in the Porous Permeable Titanium Nickel Based Alloy Scaffold

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Abstract

The respond of the cells to electromagnetic radiation depends on many factors, including microenvironment. Different cells exhibit different behavior when exposed to low-intensity radiation employed in the research. The effect of infrared (IR) and ultraviolet (UV) radiation leads to the reliable change in the number of viable cells. Viable cells of Ehrlich tumor, spleen and bone marrow of C57BL/6 mice placed in a porous permeable TiNi-based alloy scaffold were studied. The level of the radiation effect was determined for different types of cells. A comparative analysis of the viable cells cultured in the porous permeable TiNi scaffold was performed.

1 Introduction

Methods of the long-term culture of cells, including predecessor cells of special tissues, create the precondition for development of new technologies of the cell and tissue therapy. The construction of bioartificial organs is an important methodological step in transplantation of cultured cells in the damaged area.

Successful treatment depends on the number of effect of cells in the defect area. After that, cells adhere the cultured cells to the tissues, maintaining functionality. While developing the transplant technology of *in vitro* cultured cells, it is important to provide optimal conditions for the recipient area. Another problem to tackle is

long-term conservation of the functional activity of the cells implanted *in vivo*. Introduction of the predecessor cell suspension is ineffective; therefore, a carrier for cell transplantation in the recipient organism is to be found.

The approach used for cell and tissue engineering makes possible to produce new composite materials to restore the dysfunction of individual tissues or organs as a whole. The basic principles of this approach imply development and application of implantation in the damaged organ or tissue. The carrier for different biomaterial in combination with donor cells and/or bioactive substances can also be used.

The implant material used for manufacturing the structures should have multifunctional properties such as: elasticity and mechanical strength; biocompatibility at the protein and cellular level; ability to create the conditions for cell adhesion stimulating cell proliferation and differentiation; possibility of sterilization with no change in medical and technical properties.

Porous-permeable TiNi-based alloy scaffolds have been actively used over the last years to design biocompatible scaffolds for regeneration of the glands and liver tissues damaged by tumors and other etiologies, manufacturing blood vessel implants, and closing defects of soft and bone tissues.

The human body is an open thermodynamic system. The body can absorb and release electromagnetic energy. The tissues of the body respond to any energy changes at the cellular level. The radiation effect on the cell biological system depends on numerous environmental factors (wavelength, radiation energy density and pulse duration). Many factors have a significant impact on cell populations of the organism, and the factors of the intercellular space and the environment are among them.

Electromagnetic radiation is the most complicated and important physical factor that affects cellular processes. The biological area is constantly changing under various types of radiation effects occurring between both cells and tissues and in the biosphere. Insignificantly changed irradiation parameters can change the respond of cells up to their death.

Different types of radiation modify the state of the bilipid cell membrane, thus increasing or decreasing penetration of water into the cell cytoplasm and hence penetration of ions and dissolved substances. With this, polarization of the cell membrane changes, and consequently, receptors and signal system of the cell are

modified. These effects can be clearly observed at amplified intensity of natural light when blood circulation in capillaries and vessels accelerates [1-3].

When the tissue is exposed to IR radiation, radiation is absorbed by water molecules, oxygen, enzymes, cell membranes and other structures. The heat released as a result of IR exposure increases the vibration energy of the molecules and changes the processes in the entire thermodynamic system of the body. IR irradiation increases the biological activity of cells, accelerates blood circulation, increases the activity of glands, and decreases muscle spasms and pain syndrome.

UV radiation changes the properties of biopolymers (proteins and nucleic acids). Biopolymer molecules contain ring groups that intensively resonate and absorb radiation. This energy can be transmitted through the chain of atoms within the molecule without substantial loss until it reaches a weak bond between atoms and breaks it. During this process (photolysis), particles of molecules, radicals and ions are formed and damage the cell structures. An example is denaturation of proteins caused by UV radiation.

As a result, electromagnetic exposure (IR, visible and UV radiation) has a different effect on cells. It depends on many factors, including cellular microenvironment. The level of the radiation effect on the cell biosystem depends on numerous parameters (wavelength, radiation energy, and duration of the process) [2, 4].

2 Experimental

The effect of electromagnetic waves of different ranges (low-intensive) on cells of various biological tissues (spleen cells, bone marrow and Erlich adenocarcinoma) was studied. The cells were immobilized in a porous-permeable TiNi-based scaffold (Fig. 1). The shape of the scaffold depends on the method and place of implantation.





Fig. 1. The view of the experimental forms of the porous-permeable carrier of cell culture of TiNi based alloy: a – the thin disks have the size (1mm×30 mm); b – the parallelepipeds (4 mm×4 mm×10 mm); c – the cylinders for the endoscope method of the trocar implantation (5 mm×10 mm); d – the finely granulated porous-permeable crumbs (from 1 μm - 1mm)

The material for scaffolds is biocompatible (biochemical and biomechanical compatibility) at the cellular level and is in compliance with the medico-technical requirements for implant materials. The main properties of TiNi scaffolds are lack of a carcinogenic effect, antigenicity and mutagenicity, similarity between mechanical deformation and natural tissues (hysteresis response to the external influence), high permeability and wettability with tissue fluids and the possibility of sterilization by various methods with no change in functional properties [1].

This material exhibits a wide range of porous-permeable structure states required for culture of different cells. The porous samples used had a porous structure (about 70%) with pore sizes ranging from 0.1 μm to 1000 μm . The size of the pores depends on scaffolds used for different tissues.

Finely granulated porous-permeable TiNi alloy with the granule size ranging from 1 μm to 100 μm and scaffolds of porous TiNi alloy were used in the experiments.

The topography of the sample surface and the structure of the porous area were studied using a scanning electron microscope Quanta 200 3D.

The cellular material (bone marrow and spleen cells) was obtained from C57BL/6 mice. The tumor cells of Ehrlich carcinoma (ascites version) were isolated from the ascitic fluid of C57BL/6 mice. All the cells were cultured in a complete culture medium consisting of RPMI-1940 medium supplemented with 10% embryonic calf serum, 250 mg/l glutamine and 0.04 mg/l gentamicin.

The cells required for culture were seeded in a 96-well plate. The plate was placed under the LEDs and irradiated at a distance of about 10 mm above the liquid

surface. The cells were cultured in the incubator at 37°C and 100% humidity for the necessary time period. After culturing, the cell viability was evaluated by the trypan blue dye method. Each of the LEDs irradiated three wells filled with cell suspension. The sterile samples of the finely granulated and porous-permeable TiNi-based alloy (2.5 mm×2.5 mm×2 mm) were added to all experimental wells. The samples occupied about 1/10 of the culture volume.

Control groups for comparison:

- Control – cell suspension cultured when exposed to artificial radiation (daylight);
- Control+TiNi – cell suspension cultured when exposed to artificial radiation (daylight) with the samples of TiNi-based alloy;
- IR – cell suspension after exposure to IR radiation;
- UV – cell suspension after exposure to UV radiation;
- IR+TiNi – cell suspension with the samples of TiNi-based alloy after exposure to IR radiation;
- UV+TiNi – cell suspension with the samples of TiNi-based alloy after exposure to UV radiation.

Cells and samples in the incubators were irradiated by the infrared(L-53SF6C) and ultraviolet (LLT-UVLED11) LEDs for 4 hours at the radiation power of 4–6 mW/cm², followed by the adaptation period of 20 hours. After 30-min treatment with 0.25% trypsin-EDTA, the TiNi samples were removed and the plates were centrifuged. The cell suspensions were resuspended, and the number of viable cells was calculated using 0.4% trypan blue. Then the percentage of the trypan-negative cells was calculated relative to the total number of cells and the obtained data was statistically analyzed [4-6].

3 Results and discussion

The study of the IR and UV radiation effect on Ehrlich tumor, spleen and bone marrow cells of C57BL/6 mice showed the radiation effect on cells irradiated for 4 hours (radiation intensity was about 40 W/m²).

Exposure of the tested cells to IR radiation of low intensity increased the number of viable cells compared to that of non-irradiated cells. Exposure to UV radiation significantly decreases the viability of cell populations (Fig .2).

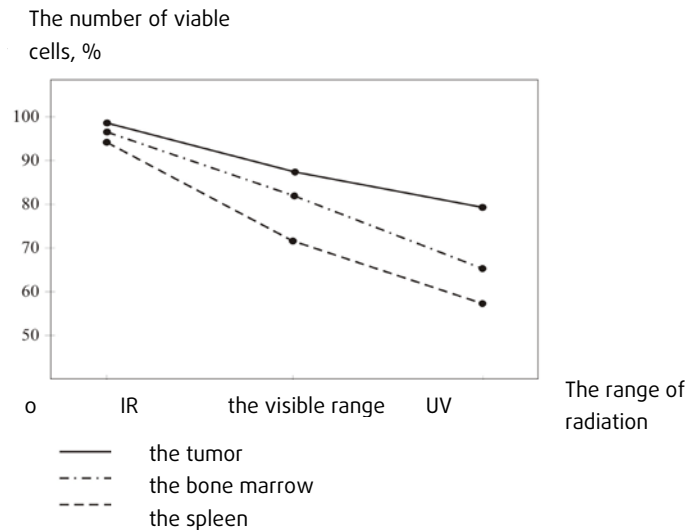


Fig. 2. The viability of cells (the tumor of Ehrlich, the spleen and the bone marrow) in during effect of IR and UV radiation after 4 hours

Exposure of bone marrow, spleen and tumor cells to low intensive radiation showed that stem cells of the bone marrow are the most sensitive population. Insignificant increase in the number of viable cells is observed under IR radiation (920 μm). In the case of UV radiation (280 μm), the number of all viable cell populations decreases.

The cellular microenvironment and the depth of cell locations are crucial to protect the organism from various types of radiation. In our study, we attempted to describe the IR and UV radiation effect on cell suspensions. Fig. 4 illustrates the results of the IR and UV radiation effect on Ehrlich tumor, spleen and bone marrow cells of mice in the presence of the finely granulated TiNi alloy (Fig. 3).

The exposure of the tested cells to low intensity radiation in the presence of the finely granulated TiNi alloy shows that different cell cultures respond differently to the IR and UV radiation. The effect of IR radiation leads to the increased population of the bone marrow cells in the presence of the finely granulated TiNi alloy ($p < 0.05$). Exposure to UV radiation under similar conditions results in the decreased viability of all cell cultures ($p < 0.05$).

The performed analysis proved that the bone marrow cells are most sensitive to radiation. This may be due to the cell adhesion ability and faster transfer of energy effects from the material surface. In addition, the IR and UV radiation effects on cells in the presence of the finely granulated TiNi alloy differed from those observed in TiNi-based scaffold. The viability of all types of the cells decreased

under exposure to UV radiation in the presence of the finely granulated TiNi alloy. The effect of UV radiation on the cells immobilized in the porous scaffold was opposite.

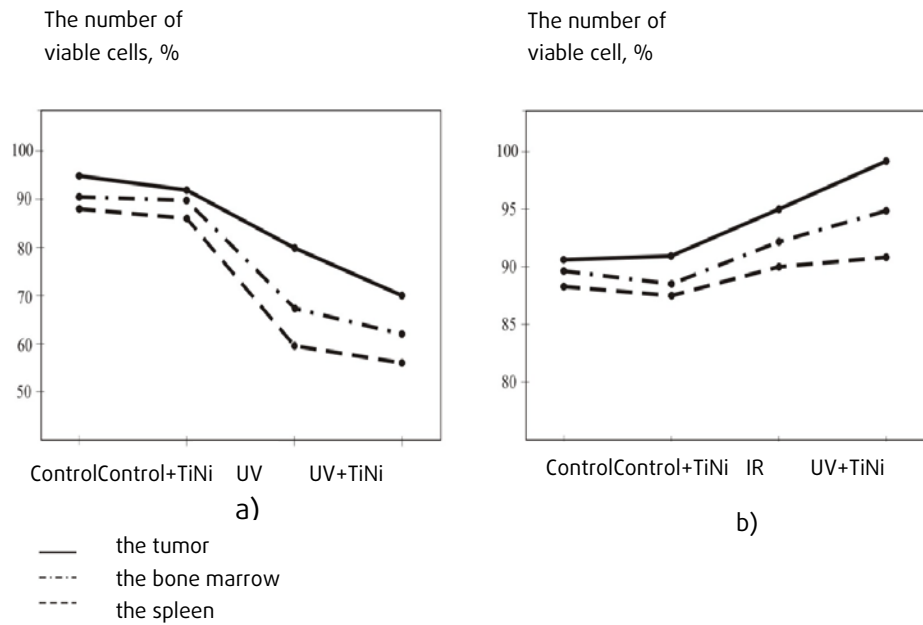


Fig. 3. The number of viable cells with the use of the finely granulated porous-permeable crumbs of TiNi alloy in the moment of the irradiation: a – the range of UV; b – the range of IR

A unidirectional effect of the IR and UV radiation was achieved in the experiment with TiNi-based scaffolds, namely the number of viable bone marrow and tumor cells increased and the number of spleen cells increased insignificantly (Fig.4).

IR spectrum promotes a 1.5-fold increase in the number of viable bone marrow cells in porous TiNi-based scaffolds. UV spectrum does not exhibit significant effect on the bone marrow cells. Both types of radiation definitely ($p < 0.05$) increase the percentage of viable tumor cells in TiNi-based scaffolds: 1.7-fold increase under UV radiation and 2-fold increase under IR radiation. The number of viable spleen cells changes insignificantly due to the predominance of differentiated cells incapable of proliferation.

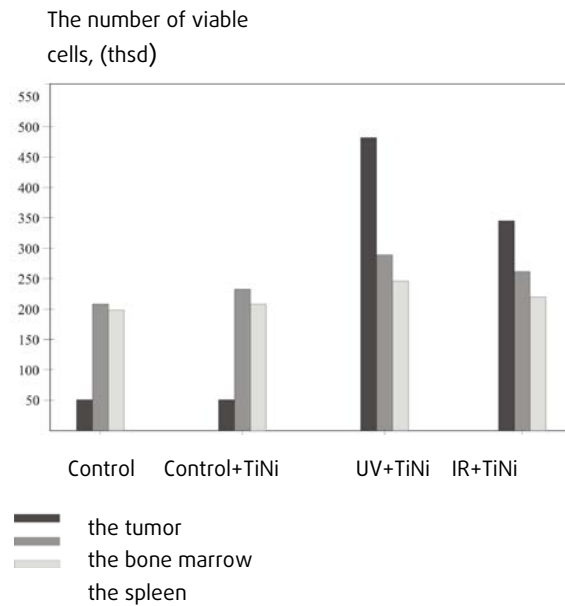


Fig. 4 The dependence of the number of viable cells in different conditions: *Control* – the nutrient medium; *Control+TiNi* – the incubator of porous TiNi without irradiation; *IR+TiNi* – the incubator of porous TiNi after infrared irradiation; *UV+TiNi* – the incubator of porous TiNi after ultraviolet irradiation

This response of cells to different types of radiation is due to transformation of the spectrum energy into the heat energy of porous TiNi-based scaffolds. At the same time, the heat energy is smoothed in the water environment of the scaffold, that is the “soft” temperature gradient affects the cell proliferation. Hence, IR and UV radiation affects the porous structure of the scaffold, which heats the pore walls and its matrix base. The liquid environment prevents its heating up to the critical (for cells) temperature of 43–45°C due to the permeable structure of the scaffold. This has a favorable effect on the stem and tumor cell proliferation. Moreover, the porous TiNi-based scaffold creates a shield to protect cells from direct UV radiation.

4 Summary

The obtained results show that exposure to IR and UV radiation enables control of the quantity and viability of cell populations. IR radiation allows increase in the viability of cells and UV radiation decreases the cell viability.

In porous permeable TiNi-based scaffolds, intercellular environment includes populations of bone marrow, tumor and spleen cells. The quantity and viability of

cell populations can be controlled by changing the parameters of the cell interaction with the external environment

The IR radiation effect can be used to resuscitate cell populations after their isolation from tissue structures and to increase the number of small population to the desired quantity. UV radiation can be used to destroy residual tumor lesions or other pathological cell populations, with the finely granulated TiNi alloy used to improve the effect of treatment.

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