DOI 10.1007/s10527-020-09953-x
Biomedical Engineering, Vol. 53, No. 6, March, 2020, pp. 407-412. Translated from Meditsinskaya Tekhnika, Vol. 53, No. 6, Nov.-Dec., 2019, pp. 24-29.
Original article submitted September 25, 2019.

Effects of Low-Temperature Plasma Glow Discharge on the Proliferative Activity of Cells and the Repair Functions of Tissues in Animals and Plants

M. Kh. Ashurov[1], S. V. Belov[2]*, S. V. Gudkov[2], Yu. K. Danyleiko[2], A. B. Egorov[2], V. V. Savranskii[2], and A. A. Temnov[3]

We present here the results of studies of the effect of low-temperature plasma on the proliferative activity of cells and the repair functions of the biological tissues in animals and plants. The influences of low-temperature plasma on the viability and proliferative activity of human stem cells are considered. Human stem cells can undergo apoptosis or proliferate, depending on the location of the zone of action and the observation time. Treatment of plants with the low-temperature plasma-activated drug Plazmolit, using dilutions giving a high concentration, was found to suppress growth and development, while low concentrations activated growth. These study results led to the conclusion that there is a single mechanism for the processes whereby low-temperature plasma affects the proliferative activity of cells and the repair functions of biological tissues in animals and plants.

Introduction

Advances in contemporary medication-based therapy, despite a constant trend towards safe and highly selective drugs, are clouded by increases in various side effects. The creation of highly selective medicines for targeted therapy and the synthesis of novel forms of stimulating formulations are accompanied by the appearance of concomitant adverse effects [1, 2]. In medicine, these are side reactions of the body, including overreactions of the immune system, individual intolerance, and initiation of the appearance of numerous multiresistant strains of pathogenic microorganisms. These manifestations have significantly spurred the search for physical factors with safe influences on the proliferative activity of cells and the repair functions of tissues in biological objects. Increasing demands of repair biology and medicine also promote the need for studies of the potential for controlling growth and differentiation of stem cells, healing processes, and tissue regeneration using physical treatments excluding many of the negative aspects of medication-based treatment. The number of studies of the effects of physical factors and identification of regimes for their targeted actions on repair and regeneration processes has now increased significantly. A considerable proportion of these studies address the effects of low-temperature plasma at the tissue and cellular levels [3-6]. The main mechanism of action of low-temperature plasma on the proliferative activity of cells and the repair and regeneration process of tissues in living organisms consists of disruption to the thermodynamic equilibrium, inducing release of calcium ions from intracellular depots, with propagation of waves of increased calcium ion concentrations in the cell cytosol, triggering calcium-dependent processes. Depend-ing on the dose, actions are associated with secondary effects consisting of a set of adaptive and compensatory reactions arising in the tissues, organs, and whole body. These reactions include activation/suppression of cellular metabolism and the corresponding increases or decreases in their functional activity, stimulation of repair processes, and anti-inflammatory action [6-8]. As regards objects of plant origin, it is logical to suppose that cell proliferation and differentiation, along with activation of plant growth and viability processes, may be mediated by the actions of

[1] GNPP Fonon, Tashkent, Republic of Uzbekistan.
[2] Prokhorov General Physics Institute, Russian Academy of Sciences, Moscow, Russia; E-mail: ser79841825@yandex.ru
[3] Sklifosovsky Research Institute of Emergency Medicine, Moscow, Russia.
* To whom correspondence should be addressed.

0006-3398/20/5306-0407 © 2020 Springer Science+Business Media, LLC

analogous physical factors [9]. Another aspect of the indirect effect of low-temperature plasma (LP) on activation of repair processes in objects of plant origin is the result of the actions of aqueous solutions of salts activated by high-frequency plasma glow discharges. Activation of solution occurs as a result of plasma burning in aqueous solution of a strong electrolyte at low concentration. Activated aqueous solutions are generally produced using a device whose operating principle is based on electrochemical methods [10]. In our case, the means of activation is fundamentally different from the traditional, allowing production of solutions with significantly different properties. In particular, the oxidative-reductive potential of such a solution can have values ranging from –1000 to +1500 mV at the moment of preparation. In addition, the process of activation of an aqueous solution by LP forms a significant quantity of peroxide [11], which increases its biological activity. A biologically activated preparation obtained using LP from a high-frequency glow-type electrical discharge was given the commercial name Plazmolit. The technique for producing Plazmolit was developed at the Prokhorov General Physics Institute, Russian Academy of Sciences [12, 13].

The aims of the present work were to assess the possibility that high-frequency glow discharge plasma has direct or indirect effects on the proliferative activity of stem cells and repair functions in plants and to evaluate the efficacy of Plazmolit using the results of treatment of agricultural plants. Test objects for studies were the simplest and most convenient objects of plant and animal origin.

Materials and Methods

The effects of glow discharge LP on the proliferative activity of cells and the repair functions of tissues were studied using the following investigations:

– the effects of LP on the viability and proliferative activity of stem cells were investigated;

– experimental assessment of the efficacy of the action of aqueous solution activated by low-temperature plasma on the rate of growth and development of agricultural legumes was performed.

1. Investigation of the Effects of Low-Temperature Plasma on the Viability and Proliferative Activity of Stem Cells

The tasks addressed were to evaluate the effects of LP on:

1) the morphology of human bone marrow stem cell cultures;

2) the proliferative activity of human bone marrow stem cells;

3) the growth factor of human bone marrow stem cells.

The experimental part of the study was carried out at the Laboratory for Cellular and Physicochemical Medical Technologies, Sklifosovsky Research Institute of Emergency Medicine.

1.1. Experimental techniques. Experiments were performed on human bone marrow stem cell cultures obtained from a healthy donor. Stem cells were prepared by a standard method using a Lympholyte-H kit for extraction (Cedarlane Laboratories Ltd., Canada). A suspension of mononuclear cells was then prepared and seeded for cultivation in Petri dishes. Experiments in Petri dishes were run by forming cell monolayers. The initial state of the cells in the monolayer was photographed using a Ti-80 inverted microscope with a Nikon microcamera. An electrode was positioned in the Petri dish at an angle such that it was inserted into the medium and was 0.5 mm from the bottom. Power in the electrode circuit was gradually increased, generating LP at the electrode tip for 9 s. The electrode was removed and the site of action was photographed. The source of the high-frequency LP was an experimental example of a specific device for dosed exposure of biological tissues to pulse-modulated radio frequency irradiation as described by the authors previously [14]. The device generated a high-frequency current with a carrier frequency of 2.64 MHz and initiation of a low-temperature plasma process in the electrolyte medium when operating in patient periodic pulse mode with pulse initiation frequencies of 10-50 kHz. Production of LP at the tip of the needle electrode was seen when the voltage of the pulse component of the high-frequency current was increased to 250 V and was accompanied by conversion to a series of short (10 ns) discharges.

1.2 Assessment of stem cell vital activity factors. Morphological evaluation of the effects of LP on blood cells was by studying lymphocyte suspensions at a concentration of $1 \cdot 10^6$ cells/mL placed in a Petri dish supplemented with vital dye trypan blue at a final concentration of 0.5%. The LP discharge lasted 3 s. The zone of action was photographed directly before and after exposure, and at 1 and 3 days.

The proliferative activity of human bone marrow stem cells was assessed using a standard method employing the MTT test, which is based on the ability of mitochondrial dehydrogenases to convert water-soluble formazan, which crystallizes within cells. Formazan was dissolved using organic solvents to provide accurate comparison of changes in the optical density of the solution as compared with controls for changes in the number of viable cells.

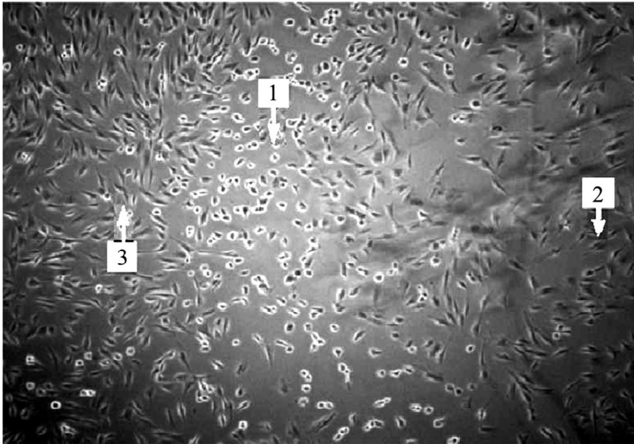

Fig. 1. Bone marrow stem cell cultures three days after exposure to LP: zone 1 – ring of "rounded" cells; zone 2 – central area of action of plasma; zone 3 – area of increased proliferation.

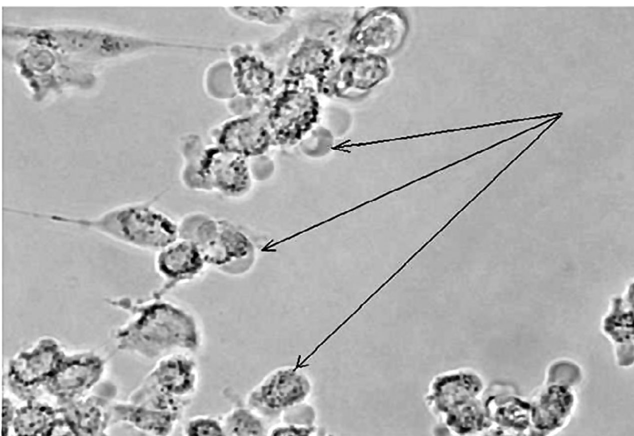

Fig. 2. Photomicrograph of the "rounded" cell zone. Black arrows show plasma membrane vesicles in the zone of action of reactive oxygen species.

Fibroblast growth factor (FGFb) is linked with a multiplicity of physiological and pathological processes, including embryonic development, neuron growth, angiogenesis, and neoplastic transformation. Basic fibroblast growth factor was assayed by ELISA in serum, plasma, or culture medium.

1.3 Study results. Exposure to LP was followed by a stepwise process with changes in cell cultures (Fig. 1). At the first stage, rings of cells formed (zone 1), which acquired a spherical shape – the "rounding-up" effect. Zone 1 expanded over a period of three days, while the central area of LP action (zone 2) remained unaltered. The peripheral part showed active stem cell growth (zone 3).

Detailed assessment of the central area (zone 2) using vital stains showed that the cellular elements were the ghosts of dead cells lacking membranes. This allows us to make the confident suggestion that degradation of the cell membrane occurs in response to reactive oxygen species produced in response to plasma. This suggestion is also supported by microphotography data obtained from the "rounded" cell zone. As shown in Fig. 2, membrane damage was seen with formation of vesicles.

The involvement of reactive oxygen species in the process of cell death was confirmed by running an experiment in which a suspension of stem cells was placed in a semiliquid medium to exclude mixing processes. This was carefully overlaid with a liquid medium in which an electrode was positioned to create an LP. Photographs of cell suspensions made at different time points after exposure to LP are shown in Fig. 3. These data confirm that the initial zone of cell death expanded over a period of 1-2 days, evidently associated with membrane lipid peroxidation and death of cells due to osmotic shock.

Studies of the effects of LP on the production of growth factors showed that exposure to LP led to the production of reactive oxygen species by human stem cells in suspension. LP was found to increase FGF production by 15% compared with intact controls, which in turn could induce increased proliferation and tissue regeneration at the whole-body level.

Conclusions. 1. The action of LP directly beneath the electrode induced rapid cell death, with formation of a zone of apoptotic cell death, which increased over a period of 1-2 days. The cause of apoptotic processes is evidently the production of reactive oxygen species occurring in response to exposure to plasma.

2. At greater distances from the electrode, increased production (or release) of FGF by bone marrow stem cells occurred, which in turn led to an increase in proliferation and tissue regeneration at the whole-body level.

3. The highest level of proliferative stem cell activity was seen at the periphery of the zone of action of LP. The cause of this increase evidently also consisted of reactive oxygen species, combined with increased FGF production.

2. Assessment of the Effects of Activated Aqueous Solution on the Rate of Growth and Development of Agricultural Plants

There are as yet virtually no evidence-based studies following the responses of different types of biological

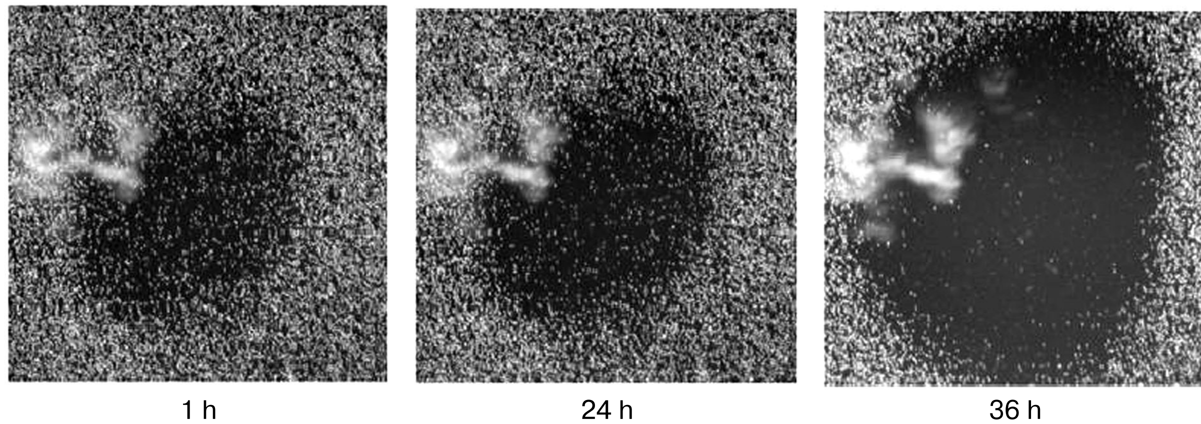

Fig. 3. Effects of LP on proliferative activity in human stem cell cultures. The proliferative activity of intact cultures was taken as 100%.

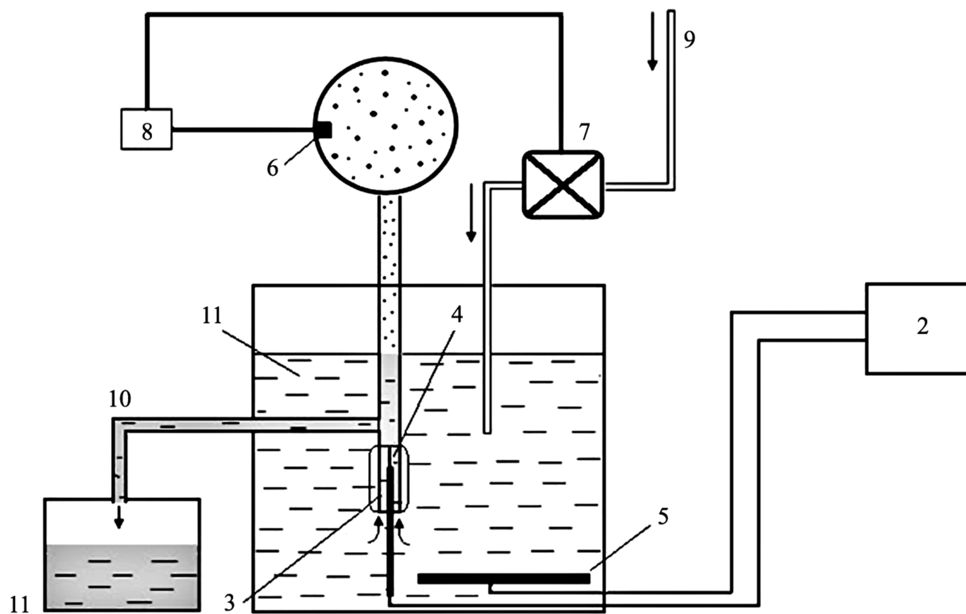

Fig. 4. Functional diagram of apparatus for preparing Plazmolit: 1 – vessel containing electrolyte solution; 2 – high-frequency generator; 3 – working cell; 4 – active electrode; 5 – neutral electrode; 6 – hydrogen probe; 7 – mixer; 8 – controller; 9 – water delivery tube; 10 – outflow tube; 11 – Plazmolit collection vessel.

objects to the action of Plazmolit. The efficacy of plasma-activated aqueous solution on the growth and development of agricultural plants was assessed using aqueous Plazmolit solution. Experiments were performed on the germination of bean seeds. Seeds sown in soil were treated with aqueous Plazmolit solution at different dilutions. Analysis of measures of the efficacy of Plazmolit action were performed at different stages of development of the plants.

2.1. Apparatus for preparation of activated aqueous solution. The experimental apparatus for preparing the biologically active preparation Plazmolit consists of an electrochemical cell with two electrodes immersed into the electrolyte, a high-frequency generator, a programmable sensor for monitoring the quantity of hydrogen released, a controllable mixer, and a working vessel. A functional diagram of the device is shown in Fig. 4.

Fig. 5. Phase of development of soybean shoots after irrigation with Plazmolit solution at different dilutions: 1 – control plants; 2 – dilution 1/100; 3 – dilution 1/500; 4 – dilution 1/2000.

The apparatus operates as follows. An aqueous solution of a strong electrolyte at low concentration (0.9-1.0% NaCl solution) is transferred from vessel 1 to working cell 3, where Plazmolit is generated by burning of low-temperature plasma in the electrolyte solution. Burning of the plasma occurrs on steel active electrode 4 in response to a high-frequency current produced by generator 2. The high-frequency current circuit is closed by electrolyte 1 in neutral graphite electrode 5. The quantity of hydrogen released is monitored using hydrogen probe 6 graduated using a calibration curve, which sends a signal to electrode controller 8 driving mixer 7. Mixer 7 is supplied with distilled water via tube 9, to enter the vessel containing electrolyte as it evaporates. Treated Plazmolit solution with specified *Ph* and *Eh* is passed to vessel 11 via collecting tube 10. Generator 2 is a powerful high-frequency generator operating at a frequency of 110 kHz, with a dynamic monitoring system for current and voltage to identify the ignition phase and the stable burning phase of the electrolyte plasma. Electronic controller 8 provides for assessment of the peroxide concentration in the treated solution in terms of the amount of hydrogen released. The system controlling the output parameters of the high-frequency generator allows the ignition process to be optimized and the plasma burning to be stabilized.

2.2. Results of treatment of agricultural crops with aqueous solution of Plazmolit. The main aim of the experiments was to obtain quantitative measures of the efficacy of the action of Plazmolit on the growth and development of soybean shoots. Treatment results were analyzed at the early stages of development using morphological tests. These addressed:

a) the number of seeds germinating;

b) entry of plants into sequential developmental phases in terms of the number of plants demonstrating appearance of the first leaf;

c) development of the root system;

d) the number of viable plants at specified experimental time points.

The results of the tests obtained from soybean seeds (Sevinch variety) planted in soil irrigated with aqueous solution of Plazmolit at different dilutions are presented in Figs. 5 and 6.

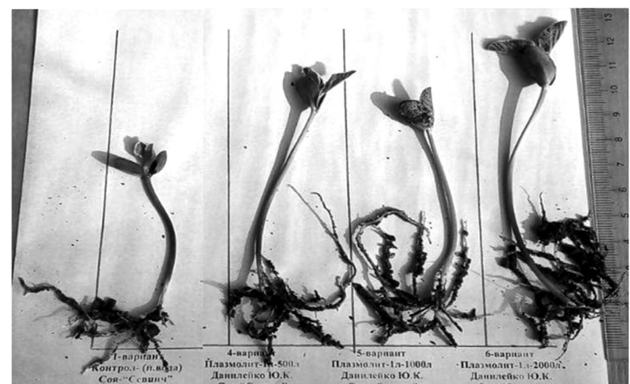

Fig. 6. Phase of development of soybean root system after irrigation with Plazmolit at different dilutions: 1 – control plants; 2 – dilution 1/500; 3 – dilution 1/1000; 4 – dilution 1/2000.

The results lead to the following conclusions:

1. Plazmolit solution can be identified as a plant growth regulator. Its use at high concentrations partly or completely blocked plant growth and development. Use of solution at lower concentrations, at dilutions of 500 or more, stimulated plant growth. Selection of the optimum concentration for a specific type of technical processing of agricultural plants will provide significant increases in yields and resistance to the influences of adverse factors.

2. For activation of plant growth at low concentrations, Plazmolit solution remained active for a long period, supporting its use as a ready preparation for both protected ground and field conditions. For this reason, Plazmolit can be used in different areas of agriculture where activated water is traditionally used.

Conclusions

Results from studies of the effects of low-temperature glow discharge plasma on the cellular proliferative activity and repair functions in biological tissues of animal and plant origin provided evidence of a single fundamental mechanism for these processes. This is supported by similarities between the results of treatment of plants with different Plazmolit concentrations and local application of different doses of LP to human bone marrow stem cells:

1) apoptosis of cells of animal origin in the zone of action of LP and suppression of plant growth and development at high concentrations of the biologically active formulation Plazmolit;

2) reversible changes in stem cells and plant growth and development processes in the limited zone of action of LP and moderate concentrations of the biologically active formulation Plazmolit;

3) activation of the proliferative activity of stem cells in the peripheral zone of action of LP and activation of plant growth at low concentrations of Plazmolit solution.

These patterns in the biology of the regenerative functions of plants and animals provide grounds for extending the selection of study objects, including use of study objects simpler than animals.

This study was carried out at the Prokhorov General Physics Institute, Russian Academy of Sciences, with partial support from the science research project “Physical methods in agriculture and ecology” (No. 0024-2019-0004). The authors would like to thank colleagues at the Laboratory for Cellular and Physicochemical Medical Technologies, Sklifosovsky Research Institute of Emergency Medicine, and colleagues at GNPP Fonon and the Institute of Rice Cultivation (Republic of Uzbekistan) for taking part in the experimental studies on test objects.

REFERENCES

1. Galeeva, G. Z., Samoilov, A. N., and Rascheskov, A. Yu., “The importance of multiresistant strains of pathogenic microorganisms in ophthalmological practice,” Vestn. Oftal’mol., **131**, No. 2, 110-114 (2015).
2. Yakovlev, S. V., “Current approaches to antibacterial treatment of hospital infections,” Lech. Vrach, No. 9, 2-5 (2005).
3. Vasil’eva, T. M., “Plasma chemical technology in biology and medicine: The current state of the problem,” Tonk. Khim. Tekhnol., **10**, No. 2, 6-9 (2015).
4. Hussein, M. U. and Yousif, E. S., “Mechanism of platelets stimulation by non thermal plasma,” Int. J. Chin. Med., **2**, No. 1, 1-5 (2018).
5. Hirschberg, J., Loewenthal, L., Krupp, A., Emmert, S., and Viöl, W., “Plasma induced changes in human lipid composition as revealed through XPS-analysis,” Natural Science, No. 8, 125-137 (2016).
6. Vasilets, V. N., Gutsol, A. F., Shekhter, A. B., and Fridman, A., “Plasm medicine,” Khim. Vysok. Energ., **43**, No. 3, 276-280 (2009).
7. Fridman, G., Friedman, A., Gutsol, A., Shekhter, B., Vasilets, V., and Fridman, A., "Applied Plasma Medicine,” Plasma Process. Polym., **5**, 503-533 (2008).
8. Dobrynin, D., Fridman, G., Fridman, A., and Friedman, G., “Physical and biological mechanisms of direct plasma interaction with living tissue,” New J. Phys., **11** (2009).
9. Gudkov, S., Grinberg, M., Sukhov, V., and Vodeneev, V., “Effect of ionizing radiation on physiological and molecular processes in plants,” J. Env. Radioact., **202**, 8-24 (2019).
10. Rogov, V. and Filipchuk, V., Electrochemical Technology of Water Properties Change, LSU Publishing House, Lviv (1989).
11. Van Hemmen, J. and Meuling, W. J., “Inactivation of biologically active DNA by γ-ray-induced superoxide radicals and their dismutation products singlet molecular oxygen and hydrogen peroxide,” Biochem. Biophys. Acta, **402**, No. 2, 133-141 (1975).
12. Baburin, N., Belov, S., Danyleiko, Y., Egorov, A., Lebedeva, T., Nefedov, S., Osiko, V., and Salyuk, V., “Heterogeneous recombination in water vapor plasma,” Rep. Acad. Sci., **426**, No. 4, 468-470 (2009).
13. Belov, S., Danileiko, Yu., Nefedov, S., Osiko, V., Salyuk, V., and Sidorov, V., “Specific features of generation of low-temperature plasma in high-frequency plasma electrosurgical apparatuses,” Biomed. Eng., **45**, No. 2, 59-63 (2011).
14. Belov, S., Danyleiko, Yu., Osiko, V., Egorov, A., and Salyuk, V., “A device for activation of tissue cell proliferation by dosed exposure to radio-frequency currents,” Biomed. Eng., **52**, No. 2, 80-83 (2018).