World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:6, No:7, 2012

Effect of Polarization and Coherence of Optical Radiation on Sturgeon Sperm Motility

Nikolai V. Barulin, Vitaly Yu. Plavskii

***Abstract*—**This work contains information about the influence low-level optical irradiation on sperm motility of sturgeon fish. On the basis of given and earlier received data the following conclusion has been made. Among the photophysical processes of a resonant and not resonant nature (oriented action of light; action of gradient forces; dipole-dipole interaction; termooptical processes), which are capable to cause the photobiological effects depended on such laser-specific characteristics as polarization and coherency, determining influence belongs to oriented action of light and dipole-dipole interactions among the processes studied in the present work.

***Keywords*—**sturgeon, aquaculture, fish sperm, laser, optical irradiation, sperm motility

I. Introduction

THE appearance of lasers amenable for medical applications intensified interest not only in study of the therapeutic efficacy of their radiation but also in the mechanisms of the primary photophysical processes determining the biological activity of the indicated physical factor. The most fundamental and critical questions were: are the observed effects laser-specific (i.e., dependent on such characteristics of the laser radiation as coherence, monochromaticity, polarization), or are they inherent to any nonlaser light source? The urgency of this question for researchers and developers of equipment has increased even more because of the appearance of concerning cheap high-brightness LEDs of visible region in the world market in the last years. Practically these LEDs don't concede to the laser sources used in the equipment for low level laser therapy by the integral intensity of radiation [1]. Moreover LEDs surpass them significantly in reliability.

Despite the large number of experimental studies [2-8] and theoretical estimates [9,10] on this problem, debates concerning a possible role for coherence and polarization of the optical radiation in realization of its biological effect have not slackened. One reason for the controversial nature of the problem under discussion is the availability in the literature of contradictory (sometimes mutually exclusive) actual data on the dependence of the photobiological effect on the above-indicated characteristics of the influencing factor.Thus in some papers (see, for example, [2-10], priority is given to the polarization of the radiation in realization of the biological and therapeutic effect of optical radiation. In this case, the most convincing support for a dependence of the biological activity of optical radiation on polarization was obtained under in vitro conditions with blood cells (λ = 400–800 nm, P = 40 mW/cm^2) [11] and cultured cells (λ = 632.8 nm, P = 3 mW/cm^2) [12].

N. V. Barulin is with the Belorussian State Agriculatural Academy, Gorki, 213407 Belarus (corresponding author to provide phone: +375-2233-51420; fax: +375-2233-59485; e-mail: barulin@list.ru).

V. Yu. Plavskii, is with the B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, Minsk, Belarus (e-mail: v.plavskii@ifanbel.bas-net.by).

According to the results of the indicated papers, only linearly polarized radiation has a regulatory effect; unpolarized radiation in the same dose range does not affect the structural and functional characteristics of cell membranes [11] and the cell proliferation rate [13]. In this case, the magnitudes of the stimulating effect induced by linearly polarized emission from a laser (λ = 632.8 nm) and a quasi-monochromatic LED source (λ = 630 nm, $\Delta\lambda$ = 15 nm) are practically the same. However, in a number of studies, either the absence of an effect for incoherent sources [14] or a less pronounced effect [15] is found. Data obtained on human myocardial tissues are also available indicating that laser emission (λ = 632.8 nm) induces simultaneous modification of all the studied metabolic processes in the tissue, while a heat source (λ = 630 nm, $\Delta\lambda$ = 150 nm) induces differently directed dynamics for these indices. A critical role is also assigned to monochromaticity (and the parameter linked with it: temporal coherence) in realization of a therapeutic effect from laser radiation in [16], based on the more pronounced therapeutic effect when using a helium-neon laser (λ = 632.8 nm, $\Delta\lambda$ = 0.02 nm) compared with exposure to a semiconductor laser with λ = 650 nm, $\Delta\lambda$ = 2 nm. Furthermore, the opinion has been expressed (and some experimental confirmation has been obtained) that optical radiation can exert a biological effect only if the exposed object (the cell) is situated completely within the coherence volume of the acting field (the coherence length Lcoh is comparable with cell dimensions) [8].

Thus the literature data presented suggest that under certain conditions, the biological activity and therapeutic effect of low-intensity optical radiation in the visible and IR regions of the spectrum may depend both on the degree of coherence of the radiation and on its polarization. Specific resonance and non-resonance photophysical mechanisms have also been discussed in the literature (orientational effect of light, effect of gradient forces, dipole–dipole interactions, thermooptic processes) that are capable of inducing photobiological effects, depending on the polarization and coherence of the radiation [17]. Moreover, a number of data [10] indicate that for comparable dose load, the stimulating effect does not depend on either the coherence of the optical radiation or on its polarization. Furthermore, a widely held opinion is that there is no basis for assuming a possible role for coherence and polarization in the interaction between low-intensity optical radiation and biological systems [18].

The aim of this work is the finding-out primary photophysical mechanisms of biological effects of radiation on the basis of studies of its biological activity depending on the physical characteristics of influencing factor (coherence degree, polarization and exposure time). Fish sperm have been selected as the object of influence, and different types of semiconductor lasers and also high-brightness LEDs have been taken as the source of radiation.

II. Materials and Methods

A. Spectral Characteristics of the Radiation Sources

Milt of Bester sturgeon (*Huso huso* × *Acipenser ruthenus*) were placed in a Petri dish as for exposure to optical radiation. As the radiation sources, we used:

- semiconductor laser, λ = 670 nm (red region of spectrum), $\Delta\lambda$ = 2 nm, L_{coh} ~ 224 μm, continuous regime;
- broadband white LED, λ = 420–800 nm with maxima at λ = 453 nm and 567 nm, the half-width of the main band is $\Delta\lambda$ = 130 nm, coherence length (neglecting the minor component) L_{coh} < 2.5 μm, Flowmagic BV, Monster, the Netherlands;
- helium–neon laser LGN-111, λ = 632.8 nm (red region of spectrum), $\Delta\lambda$ = 0.02 nm, L_{coh} ~ 2000 μm, continuous regime, Polyaron NPO, L'vov, Ukraine.

The average power of the radiation was monitored by an IMO-3C (Russia) power meter, measuring the average power and the energy of the laser radiation.

B. The procedure of irradiation

In all the experiments with lasers or LEDs, its radiation was defocused by a lens in such a way that the size of the light spot corresponded to the area of a monolayer of the irradiated fish sperm. In order to eliminate possible artifacts due to the inhomogeneous spatial distribution of the acting radiation, the position of the Petri dish relative to the light spot was changed every 15–20 sec without changing the distance to the radiation source. The fish sperm were irradiated for 30, 60, 90, 180, 300, 600 sec at a temperature of $16^oC \pm 1^o$ C. Control (untreated) fish sperm specimens were also placed in the Petri dish and experienced the same conditions (except for irradiation) as the test specimens.

C. Influence of optical radiation on sperm motility

The following quantities were used as parameters to characterize how radiation affects sperm motility of the fish: γ= (Mt/Mc) 100% where Mc are the sperm motility that was not subjected to laser (or LED) radiation (the control group), and Mt are the sperm motility of the individuals obtained from irradiated eggs (the test group).

D. Statistical data

Data were analyzed by one way ANOVA, Wilcoxon test, test $\chi 2$, one-sided Fisher's exact test, Mann-Whitney test and Dunnett's test. P<0.05 was considered significant. All values are means±standard error (SE).

III. Dependence of the Biological Effect of Optical Radiation on the Type of Polarization

Table I gives the quantitative data which are evidence for a difference between the photobiological effects (sperm motility), induced by exposure of the fish sperm under optimal conditions to linearly polarized and unpolarized radiation.

TABLE I
EFFECT OF IRRADIATION OF LASER AND LIGHT-EMITTING DIODE OF FISH SPERM ON THE SPERM MOTILITY

Irradiation regime	Irradiation time, sec	Sperm motility, sec
Control	0	120±7.6
Broadband linearly polarized white LED (P = 1.5±0.2 mW/cm^2, λ = 420–800 nm, $\Delta\lambda$ = 130 nm, Lcoh < 2.5 μm)	30	225±10.4*
	60	200±5.8*
	90	190±5.8*
	180	150±11.5
	300	145±13.2
Broadband linearly unpolarized white LED (P = 1.5±0.2 mW/cm^2, λ = 420–800 nm, $\Delta\lambda$ = 130 nm, Lcoh < 2.5 μm)	30	200±15.3*
	60	180±11.5
	90	170±2.9*
	180	140±2.9
	300	150±2.9
Monochromatic linearly polarized lazer (*P* = 1.5±0.2 mW/cm^2, (λ = 670 nm, $\Delta\lambda$ = 2 nm, Lcoh ~ 224 μm)	30	260±7.6**
	60	230±5.8**
	90	200±2.9*
	180	184±5.0*
	300	170±2.9*

Reliability of differences from control:
* - P<0.05
** - P<0.01

Our results showed that the laser and light-emitting diode irradiation has a high stimulating effect on sperm motility. The maximum sperm motility in the experimental group was 260±7.6 sec (group with monochromatic linearly polarized lazer, t = 30 sec), while in the control group, the sperm motility was 120±7.6 sec. Unpolarized radiation also results in significant differences from the control ($p < 0.05$). However, under the same irradiation conditions, the magnitude of the stimulating effect for radiation with natural polarization is significantly smaller with respect to all the parameters than when radiation with linear polarization is used (maximum sperm motility was 200±15.3 sec in group with broadband linearly unpolarized white LED).

Thus all the data presented, taken together, suggest substantial differences in the biological activity of linearly polarized and unpolarized light with respect to fish sperm when monitoring the sperm motility.

As we know [9, 19], important information about the primary mechanisms of photobiological processes can be obtained from analysis of the results of the effect of radiation with linear and circular polarization on biological specimens. Figure 1 shows the quantitative data reflecting the effect on fish sperm from laser radiation (λ = 632.8 nm, P = 1.5 mW/cm2, t = 30 sec) with linear, circular, and natural polarization, and the data based on monitoring the sperm motility.

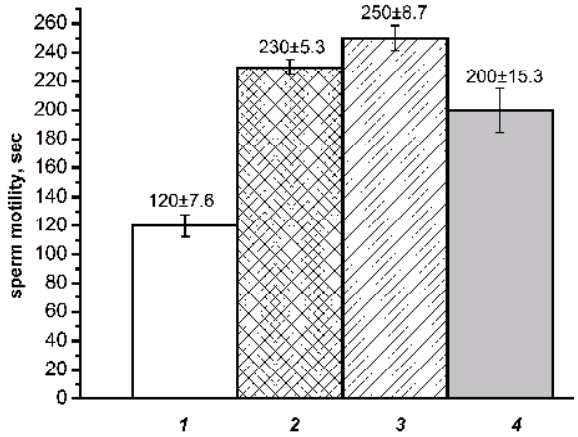

Fig. 1 Effect of the type of polarization of the laser radiation (λ = 632.8 nm, P = 1.5 mW/cm2, t = 30 sec) used to irradiate the fish sperm to sperm motility 1) control; 2) circularly polarized radiation; 3) linearly polarize radiation; 4) unpolarized radiation.

From Fig. 1 it follows that irradiation of fish sperm, affecting the sperm motility, leads to a increase (compared with the control) of the sperm motility. In this case, the maximum result (250±8.7 sec, $p < 0.05$) is observed for exposure to linearly polarized radiation; the minimum stimulating effect is observed for exposure to unpolarized radiation (200±15.3 sec, $p < 0.05$); radiation with circular polarization occupies an intermediate position with respect to biological activity (230±5.3 sec, $p < 0.05$).

According to the data obtained, significant differences are observed not only between each experimental group and the control, but also between experimental groups, which is evidence that linearly polarized, circularly polarized, and unpolarized radiation have different biological activities.

IV. Primary Photophysical Processes which Define the Biological and Therapeutic Effect of Low-Intensity Laser Radiation

It is well known that when radiation passes through biological tissue, its rapid depolarization is observed. The question naturally arises concerning how in such a case polarization of the radiation may play a critical role in realization of its biological and therapeutic effect. In this connection, we note that according to [20], light in the red and near IR regions of the spectrum can propagate in human skin to a depth of ≈1.2 mm while retaining linear polarization. Whole blood is characterized by a depolarization length of ~4.0 mm [21]. Probably the changes induced by polarized radiation within the interior of tissue (in which the polarization of the radiation is still preserved) are propagated to the body as a whole.

As already noted, a widespread opinion in the literature [10] is that neither coherence nor polarization of low-intensity radiation can affect its biological activity, since there are none of the photophysical prerequisites for this. To analyze the possible reasons for the dependence of photobiological effects on the parameters of the acting radiation, let us consider the existing points of view concerning the mechanisms for the biological activity of light.

All current hypotheses [11, 17] can be divided into two groups: photochemical and nonphotochemical (nonresonance). The authors of the hypotheses in the first group assume [10, 22] that the effect of laser radiation on metabolic processes in the body is due to photochemical reactions occurring in the body on absorption of light by endogenous photoacceptors. The following biological molecules can be considered as such acceptors:

- catalase, superoxide dismutase, ceruloplasmin (an increase in the anti-oxidant activity of the indicated enzymes, decreased in the pathological state [22]);
- cytochrome c oxidase (acceleration of electron transfer in the respiratory chain as a result of a change in the oxidation–reduction properties of the electron carrier [10]);
- hemoglobin (an increase in the local concentration of molecular oxygen as a result of photodissociation of oxyhemoglobin; a change in the oxygen-transport function of blood [23];
- nitrosyl complexes of heme proteins (liberation of NO on exposure to light from the hemoglobin complex and then binding of the NO to cytochrome c oxidase, accompanied by modulation of its activity; vasodilator effect of NO [10]);
- molecular oxygen (formation as a result of absorption of photons by O2 molecules of singlet oxygen, capable of inducing structural rearrangements of the aqueous phase);
- endogenous porphyrins (uroporphyrins, coproporphyrins, hematoporphyrins) and flavins (photosensitized formation of active forms of oxygen [10]).

In fact, based on the indicated photochemical hypothesis and considering that biomolecules have very broad absorption bands and short dephasing times for the excited vibrational states, it is difficult to expect that the photobiological effect will be laser-specific (dependent on the coherence, monochromaticity). In turn, it is determined by the number of photons absorbed by the system (the radiation dose). However, the opinion has been expressed [17] that besides photochemical processes, some role may be played in realization of the biological effect of laser radiation (especially for the pulsed exposure variant) by the optothermic effect (an increase in temperature in the vicinity of the chromophore molecule that has absorbed a photon). According to estimates in [17], the difference in the magnitude of the optothermic effect for polarized and unpolarized radiation may be the reason for the different biological response of cells to the indicated types of exposures. Along with the resonance hypothesis, a number of authors have developed the concept of nonphotochemical (nonresonance) mechanisms for the biological effect of both coherent and incoherent radiation [11], not due to absorption of photons by the components of the biological system, capable of inducing biological effects, dependent on such laser-specific characteristics as coherence and polarization. Among the indicated mechanisms, we include: gradient dipole interactions arising on exposure of the system to radiation with spatial modulation of the intensity [9]; dipole–dipole interactions induced by a light wave in nearby structures [17]; orientational effect of radiation [8].

The effect of gradient forces on biological organelles, cells, and other micron-size formations is connected for formation of a speckle structure by the laser radiation as a result of interference between the incident beam and the reflected and scattered (by tissue inhomogeneities) beams. As a result of nonresonance dipole interaction of the electrical component of light with the photoinduced dipole moment of biological microparticles, gradient forces arise that can have a biological effect, including as a result of selective increase in the kinetic energy of the microparticles [9].

In contrast to gradient forces, interaction of photoinduced oscillating dipole moments of adjacent particles with each other (dipole-dipole interaction) that is nonresonant in character (in the absence of absorption) may be realized when biological specimens are exposed to both coherent and incoherent radiation [9]. One more mechanism for the nonresonance effect of light on biological systems involves the orientational effect of radiation [11,12], inducing a change in the spatial structure of the cell components with a liquid-crystalline type of ordering, responsible for regulation of metabolic processes (macromolecules of enzymes, membranes). The indicated mechanism is the optical Kerr effect and should be observed for molecules characterized by anisotropy of the polarizability.

The photophysical mechanism for these changes is reorientation of individual highly ordered anisotropic sections (domains) of the indicated components as a result of interaction between the electric field of the light wave and the induced (by this wave) integrated electric dipole of the domain [11, 12]. The results obtained in this work and also our previous studies [24-26] allow us to conclude that the photobiological effects may be interpreted from the standpoint of a nonresonant nonphotochemical mechanism of action for the radiation. Besides a dependence of the effect on the polarization, evidence in favor of this idea comes from: a) the influence of a constant magnetic field on effects induced by linearly polarized light; b) dependence of the effect on the peak intensity of the radiation (when using pulsed nanosecond radiation – semiconductor AlGaAs laser, 890 nm, pulse duration 100 nsec), the average power density P = 0.06–0.24 mW/cm2 is at least an order of magnitude lower than the corresponding value for cw radiation); c) violation of the Bunsen–Roscoe law of reciprocity of time and power density; d) effect of the modulation frequency on the detectable photobiological effect.

V. Conclusion

It is shown that sturgeon sperm are convenient objects for estimating the biological activity of the radiation, while the presence of a photobiological effect can be confirmed by testing the sperm motility. The results obtained suggest a pronounced dependence of the biological effect of laser radiation on its type of polarization. The maximum stimulating effect on the sperm motility of sturgeon is induced by irradiation by linearly polarized light. It is hypothesized that the primary photophysical mechanisms underlying the influence of radiation on metabolic processes in the body are due to cooperative structural transitions in membranes and multiple-enzyme complexes as a result of the orientational effect of polarized radiation, and also nonresonant dipole–dipole interactions.

References

[1] V.Yu. Plavskii [et al.] (2007). Apparatus for low-level laser therapy: modern status and development trends. *J Opt Technol.* 74 (4), 246-257.

[2] E. Mester (1966). The use of the laser beam in therapy. *Orv. Hetil., 107 (22),* 1012–1016.

[3] M. Fenyö (1984). Theoretical and experimental basis of biostimulation by laser irradiation. *Opt. Laser Technol., 16 (4),* 209-215.

[4] K. Yokoyama, K. Sugiyama, (2001). Temporomandibular joint pain analgesia by linearly polarized near-infrared irradiation. *Clinical J. Pain., 17 (1),* 47–51.

[5] L. Medenica, M. Lens, (2003). The use of polarized polychromatic non-coherent light alone as a therapy for venous leg ulceration. *J. Wound Care, 12 (1),* 37–40.

[6] K. Yokoyama, K. Sugiyama (2003). Influence of linearly polarized near-infrared irradiation on deformability of human stored erythrocytes. *J. Clin. Laser Med. Surg., 21 (1),* 19–22.

[7] J. Verbelen (2007). Use of polarised light as a method of pressure ulcer prevention in an adult intensive care unit. *J. Wound Care., 16 (4),* 145–150.

[8] A.V. Budagovsky (2005). On the ability of cells to distinguish the coherence of optical radiation. *Quantum Electronics, 35 (4),* 369–374.

[9] A.N. Rubinov, A.A. Afanas'ev (2005). Nonresonance mechanisms of biological effects of coherent and incoherent light. *Opt. Spectr., 98 (6),* 943–948.

[10] T. Karu (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. *J. Photochem. Photobiol. B: Biol., 49 (1),* 1–17.

[11] M. Fenyö, J. Mandl, & A. Falus (2002). Opposite effect of linearly polarized light on biosynthesis of interleukin-6 in a human B lymphoid cell line and peripheral human monocytes. *Cell Biol. Intern., 26 (3),* 265–269.

[12] V.A. Mostovnikov [et al.] (1994). Primary photophysical processes which define the biological and therapeutic effect of low-intensity laser radiation. *Proc. SPIE, 2370,* 541–548.

[13] .Yu. Plavskii [et al.] (2007). Apparatus for low-level laser therapy: modern status and development trends. *J Opt Technol. 74 (4),* 246-257.

[14] J. Kubota, T. Ohshiro (1989). The effects of diode laser low reactive-level laser therapy (LLLT) on flap survival in a rat model. *Laser Therapy, 1 (3),* 127–134.

[15] I. Onac, L. Pop, & I. Onac (1999). Implications of low-power He-Ne laser and monochromatic red light biostimulation in protein and glycoside metabolism. *Laser Therapy, 11 (1)* 130–137.

[16] T. Qadri [et al.] 2007The importance of coherence length in laser phototherapy of gingival inflammation – a pilot study. *Lasers Surg. Med., 22 (4),* 245–251.

[17] V.A. Tolkachev (2004). Role of Light Polarization in the Optothermal Effect. *J. Appl. Spectrosc., 71, (1),* 139–142.

[18] Yu.A. Vladimirov [et al.] (2004). Molecular and cellular mechanisms triggered by low-level laser irradiation. *Biophysics (Moscow), 49 (2),* 325–336.

[19] V.Yu. Plavskii & N.V. Barulin (2008). Effect of polarization and coherence of low-intensity optical radiation on fish embryos. *J Appl Spectrosc, Vol. 75, No. 6,* pp 843–856.

[20] V.V. Tuchin (1997). Light scattering study of tissues. *Physics-Uspekhi, 40 (5),* 495–515.

[21] T.I. Karu, L.V. Pyatibrat & T.P. Ryabykh, (1997). Nonmonotonic behavior of the dose dependence of the radiation effect on cells in vitro exposed to pulsed laser radiation at λ = 820 nm. *Lasers Surg. Med. 21 (5),* 485–492.

[22] S.M. Zubkova (1978). Mechanism of biological effect of helium-neon laser irradiation *Nauchnye Dokl. Vyss. Shkoly. Biol. Nauki (Moscow), No 7,* 30–37 [in Russian]

[23] G.A. Zalesskaya [et al.] (2006). Effect of intravenous laser irradiation on the molecular structure of blood and blood components. *J. Appl. Spectrosc., 73 (1),* 115–122.

[24] V.Yu. Plavskii, & N.V. Barulin (2008). Effect of polarization and coherence of low-intensity optical radiation on fish embryos. *J Appl Spectrosc, Vol. 75, No. 6,* pp 843–856.

[25] V.Yu. Plavskii, & N.V. Barulin (2008). Effect of exposure of sturgeon roe to low-intensity laser radiation on the hardiness of juvenile sturgeon. *J Appl Spectrosc, 75 (2),* 241–250.
[26] V.Yu. Plavskii, & N.V. Barulin (2008). How the biological activity of low-intensity laser radiation depends on its modulation frequency. *J Opt Technol., 75 (9),* 546–552.