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Elementary Processes in Cells after Light Absorption Do Not Depend on the Degree of Polarization: Implications for the Mechanisms of Laser Phototherapy

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ABSTRACT

Objective: **The objective of this work was to evaluate the importance of the degree of light polarization in stimulation of cellular metabolism.** ***Background D*****ata: Although the possible role of polarization's effects on the mechanisms of laser phototherapy is sometimes discussed in the literature, there are still no clear answers.** ***Material and Methods:*** **A model system (HeLa cell suspension) was used in which the lengths of light scattering (ℓ_{sc}) and absorption (ℓ_a) were much larger than the thickness of the irradiated layer (L = 3 mm). The cell suspension (1×10^6 cells/cm^3) was irradiated with a diode laser (λ = 637 nm, D = 65.7 J/m^2, τ = 10 sec, I = 6.57 W/m^2). The polarization degree (99.4%, 60.9%, and 34.2%) of the beam was changed by means of optical fibers of different lengths. The irradiated suspension was incubated at 37°C for 30 min, and the attached cells were counted afterwards.** ***Results:*** **The cell fraction stimulated to adhere by red light at 637 nm was nearly the same in all three experimental groups (58.1% ± 2.5%, 57.6% ± 3.5%, and 62.5% ± 3.2% for degree of beam polarization of 99.4%, 66.9%, and 34.2%, respectively). There was no statistically significant difference in these results (p <0.8, <0.6, and <0.7, respectively). At the same time, all three groups had statistically significant differences ($p < 0.01$) in adherence from the sham-irradiated control group (39.1% ± 2.2%).** ***Conclusion:*** **The biological effect (stimulation of cell attachment) of light with λ = 637 nm on cells in our model system was pronounced, but did not depend on the degree of light polarization. Elementary processes in cells (light absorption and photochemistry) do not appear to depend on the degree of light polarization.**

INTRODUCTION

LASER PHOTOTHERAPY is an effective tool for the treatment of musculoskeletal injures, arthritis, chronic wounds, and other conditions. Laser phototherapy utilizes monochromatic or quasi-monochromatic radiation in the red-to-near infrared (IR) region, both from lasers and light-emitting diodes (LEDs).

During the approximately 40-year history of this modality, a widely discussed issue in the laser phototherapy clinical community has been the question of whether the coherence and polarization of laser radiation have additional benefits, as compared to monochromatic light from a conventional light source or LED of the same wavelength and intensity. Specially designed experiments at the cellular level showed that He-Ne laser radiation (i.e., coherent and polarized light at 632.8 nm), and properly filtered light (633 ± 4 nm) from a conventional light source with the same intensity and irradiation time have the same biological effect.[1] The same conclusion was drawn from an experiment in which radiation from an He-Ne laser was used with or without an optical fiber for full depolarization of the beam.[2] The successful use of LEDs in many areas of clinical practice also confirms this conclusion. The question of the role of light coherence has also been considered theoretically.[3,4]

Only a few studies[1,2] have compared the action of polarized and nonpolarized light with other parameters remaining equal. In the work of Kubasova et al.,[5] the blast-transformation and rosette-forming abilities of human lymphocytes were studied after irradiation with diffuse and linearly polarized light. It was

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found that both types of light increased the number of blast-transformed cells and reduced the rosette-formation of T lymphocytes. However, the authors concluded, though without statistical evaluation, that linearly polarized light had a more pronounced effect.

A randomized, placebo-controlled double-blind trial was designed to study changes in the humoral immunity of a large group of volunteers after exposure of a small body area to polychromatic visible and infrared polarized and nonpolarized light (400–3400 nm, 95% polarization, 40 mW/cm^2, 12 J/cm^2, and 400–3400 nm, no polarization, 38 mW/cm^2, 11.2 J/cm^2, respectively). The authors' conclusion demonstrated the similarity of effects induced by a single exposure to both light sources.[6]

In the experiments of Bolton et al.,[7] cells of a macrophage-like cell line (U-937) in suspension were exposed *in vitro* to a light-source emitting 400–2000 nm with different levels of polarization (95% and 14%), but the same energy density. Then the macrophage-conditioned medium was removed and placed on 3T3 fibroblast monolayer. The proliferative response was greatest in the cultures exposed to supernatants from macrophages with the 95% polarization of the light source at the irradiation time of 120 sec. However, the effect of 14% polarization was also high when irradiated for 60 sec.

Chumak et al.[8] found that irradiation of rat hind limb with polychromatic polarized light at 400–2000 nm produced a long-term inhibition of baseline afferent traffic in the saphenous nerve, while nonpolarized light produced biphasic changes in baseline activity. Ribeiro et al.[9] investigated the healing of burns in rats by irradiating the lesions with a linearly polarized He-Ne laser beam parallel to the spinal column of the rat, or using the same laser and dose, but aligning the light polarization perpendicularly to the relative orientation of the spinal column. They found that skin wound repair was dependent on polarization orientation with respect to a referential axis as the animal's spinal column.

To date, there is no actual evidence regarding the special effects of light polarization for medical treatment. There is a group of papers in which linearly polarized broadband radiation (600–1600 nm) was used to treat pain.[10–12] Linearly polarized light at 830 nm was used to improve the deformability of human erythrocytes.[13] Broadband polarized radiation (400–2000 nm) was used to improve healing of deep dermal burns[14] and excision wounds[15] as well as for biological studies *in vitro*.[16] Comparison with the action of nonpolarized light was not done in these studies.

To obtain unambiguous data on the role of light polarization in laser phototherapy, a model system characterized by equal light polarization in the whole irradiated volume has to be used. This requirement is fulfilled in the cell suspension with a certain cell concentration in a rather thin layer. This model was used earlier to evaluate cell attachment increases under irradiation.[17] The same model (thickness of irradiated layer suspension L = 3 mm and counting of attached cells after a 30-min incubation period) is used also in the present work.

Our experiments aimed to study a possible dependence on degree of light polarization (99.4%, 60.9%, and 34.2% linearly polarized light at 637 nm) on the model of cell attachment to a glass matrix. We intend to show that the cell fraction stimulated to adhere is nearly equal in all three cases.

MATERIAL AND METHODS

Cells

The HeLa cells obtained from the Institute of Virology, Moscow, Russia, were cultivated as a monolayer in closed scintillation vials at 37°C in 2 mL of RMPI-1640 (ICN Pharmaceutical, Amsterdam, The Netherlands) with 10% of fetal bovine embryo serum (ICN Pharmaceutical) and 100 U/mL of streptomycin and grown 72 h (middle-log phase). The HeLa culture used is characterized by anchorage-dependent growth and forms a confluent monolayer.

Cells were harvested using warm (37°C) 0.02% Versene solution (ICN Pharmaceutical). Suspensions for irradiation were prepared in RPMI-1640 medium containing 10% bovine embryo serum. Cell culture processing was performed in darkness or under dim natural light.

Light source

A GaAlAs diode laser (Matrix; Research and Production Laser Center Matrix, Moscow, Russia) with the head ML06 (λ = 637 nm, polarization coefficient K_{pol} = 99.4%, CW radiation) was used. K_{pol} Rayon was decreased by a polymer optical fiber PMMA (Mitsubishi Co., Tokyo, Japan) 50 cm in length (K_{pol} = 60.9%) or 100 cm in length (K_{pol} = 34.2%). The homogeneous light spot covered the exposed cell suspension surface (0.28 cm^2; Fig. 1). An absence of light diffusion was checked visually outside the point of exit of light from the sample cells. The radiation intensity was the same in all experiments (6.57 W/m^2), as was the irradiation time (10 sec). The irradiation dose was 65.7 J/m^2. The parameters of the light used in this experiment were in the optimal region to promote stimulation of cell attachment in this particular cellular model.[17] Intensity measurements were made using a FieldMax II laser power meter (Coherent, Santa Clara, CA) (Fig. 1A). The laser parameters and irradiation conditions were strictly controlled during the experiments.

Irradiation

The samples of cellular suspension were irradiated in special glass sample cells (Fig. 1A). These sample cells were made as follows: Two glass rings with ground-in edges (inner diameter 0.6 cm, height 0.3 cm, volume 0.084 cm^3) were glued to a microscope slide with 5 cm of space between them. Both sample cells were filled with cell suspension (1.0×10^6 cells/cm^3). In each case, one of the sample cells was irradiated and another one was used as the control. Optimal irradiation conditions (shape and dimensions of the vial and the number of cells per vial) were ascertained in a special series of experiments.[17] Irradiation was performed at room temperature in the dark. The control sample cells were protected from light during the irradiation experiment. A new pair of sample cells was used for each measurement.

Measurement of cell-glass adhesion

The criteria by which changes in the adhesion properties of the cell membrane were judged was the number of cells that attached themselves to the bottom of the sample cell in the course of incubation for 30 min at 37° (Fig. 1B). This time period was

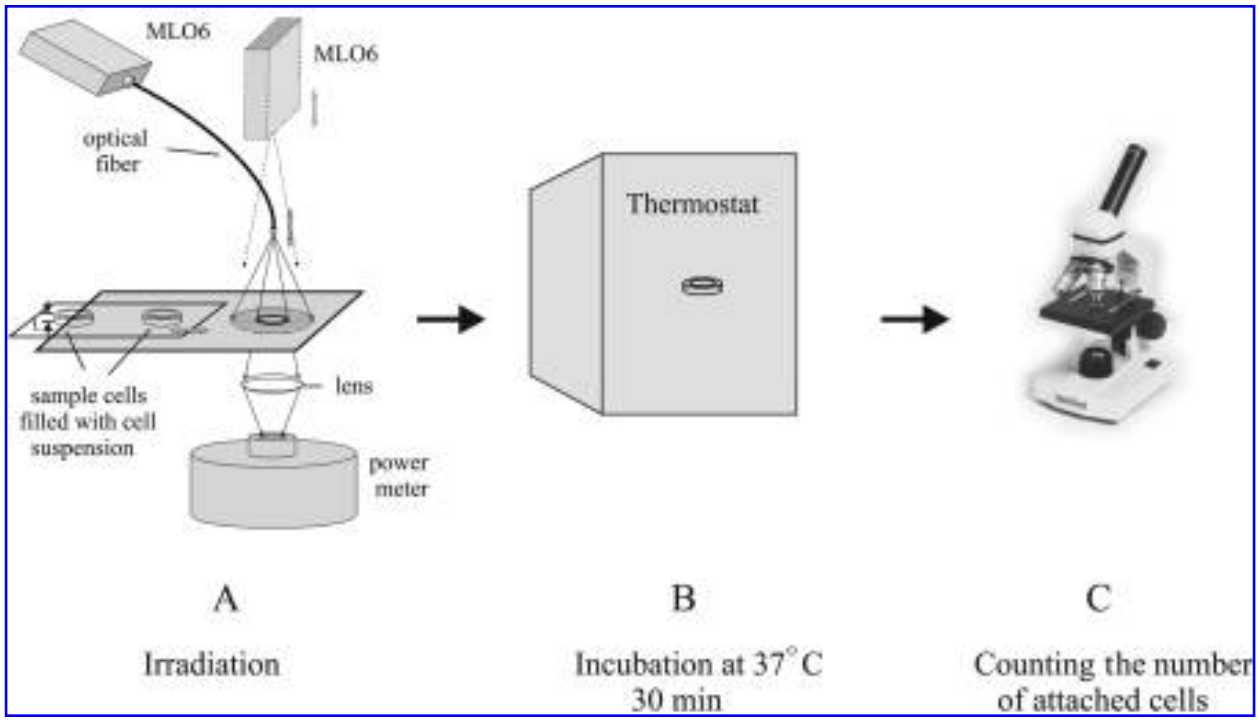

FIG. 1. Schematic of the experimental procedure.

chosen according to experiments we performed to ascertain the optimal time required for attachment of HeLa cells to the glass substrate under our experimental conditions.[17] In the control experiment, 39.1% ± 2.2% of cells adhered to the surface after 30 min of incubation. Thus, in these conditions the stimulatory and inhibitory effects of the irradiation could be measured under identical conditions. After incubation (Fig. 1B), the nutrient medium was removed, and the sample cells were washed with warm (37°) Hanks' solution to remove unattached cells. The attached cells were trypsinized, and their number was counted with a hemocytometer (Fig. 1C). Each data point in Table 1 represents the mean of 20 independent measurements.

Statistical analysis

The results obtained were statistically processed using GraphPad Prism statistical software (GraphPad Software, San Diego, CA, USA), and expressed in terms of the mean value ± standard deviation over 20 measurements for every group. The difference between the group values was evaluated by Student's *t*-test.

RESULTS

The number of cells attached to the glass surface in the sham-irradiated (control) experiment was 39.1% ± 2.2%. The number of cells attached increases upon irradiation of the cell suspension. The percentages of attached cells in three experimental groups were 58.1% ± 2.5%, 57.6% ± 3.5%, and 62.5% ± 3.2% (Table 1). The fraction of cells adhered after the irradiation increased in all three experimental groups statistically significantly ($p < 0.01$) compared with the sham-irradiated control group (Table 1).

Now the question is whether there is a statistically significant difference among the results obtained for the three exper-

TABLE 1. DEPENDENCE OF CELL ATTACHMENT ON POLARIZATION COEFFICIENT (K_{POL}) OF THE IRRADIATING BEAM WHEN OTHER PARAMETERS ARE EQUAL (D = 65.7 J/M^2, τ = 10 SEC, I = 6.57 W/M^2)

K_{pol}	*Percentage of attached cells*	*Statistical significance of difference from the control*	*Increase of the attached fraction of cells due to irradiation*	*Statistical significance of difference from the control*	*Group*
Control	39.1 ± 2.2	—	—	—	—
99.4%	58.1 ± 2.5	$p < 0.01$	19.0% ± 1.3%	$p < 0.01$	A
60.9%	57.6 ± 3.5	$p < 0.01$	18.5% ± 1.8%	$p < 0.01$	B
34.2%	62.5 ± 3.2	$p < 0.01$	23.4% ± 2.0%	$p < 0.01$	C

Table 2. Statistical Significance of the Differences between the Three Experimental Groups as Evaluated by Student's *t*-Test (K_{POL} of Radiation 99.4%, 60.9%, and 34.2%, Respectively)

	Group	
Comparison	K_{pol} *(%)*	p
A vs. B	99.4 vs. 60.9	$p < 0.8$
B vs. C	60.9 vs. 34.2	$p < 0.6$
A vs. C	99.4 vs. 34.2	$p < 0.7$

The values of fractions of attached cells for groups A, B, and C are shown in Table 1.

imental groups. Recall that the cells in these groups were irradiated with light having different polarization coefficients with other parameters being equal. This analysis is presented in Table 2. Based on these data, it can be concluded that the effect of light with $\lambda = 637$ nm on cell adherence is quite pronounced in our model system, but it does not depend on the degree of light polarization.

DISCUSSION

The results obtained in this study allow us to conclude that elementary processes in cells (light absorption, photochemistry, and photosignal transduction's biochemical reactions in a cell[18]) that occur during irradiation, as well as during the 30-min incubation period, do not depend on the degree of light polarization. This result also means that the effects of polarization are not pronounced on an elementary level when a real biotissue is irradiated with a polarized beam. However, this conclusion should be scrutinized in detail, taking into account that a cellular suspension differs from a real biotissue.

The laser beam loses its polarization during propagation in the scattering biotissue (Fig. 2). The experiments show evidence that the linear polarization in maintained for 2.5 times the so-called transport length, ℓ_{tr}.[19] The value of ℓ_{tr} depends on the reduced scattering coefficient μ'_s

$$1/\ell_{tr} = \mu'_s = \mu_s (1 - g)$$

Here g is a parameter of anisotropic scattering and μ_s is the scattering coefficient. For skin tissue in the so-called therapeutic window (the red-to-near IR optical region), μ'_s

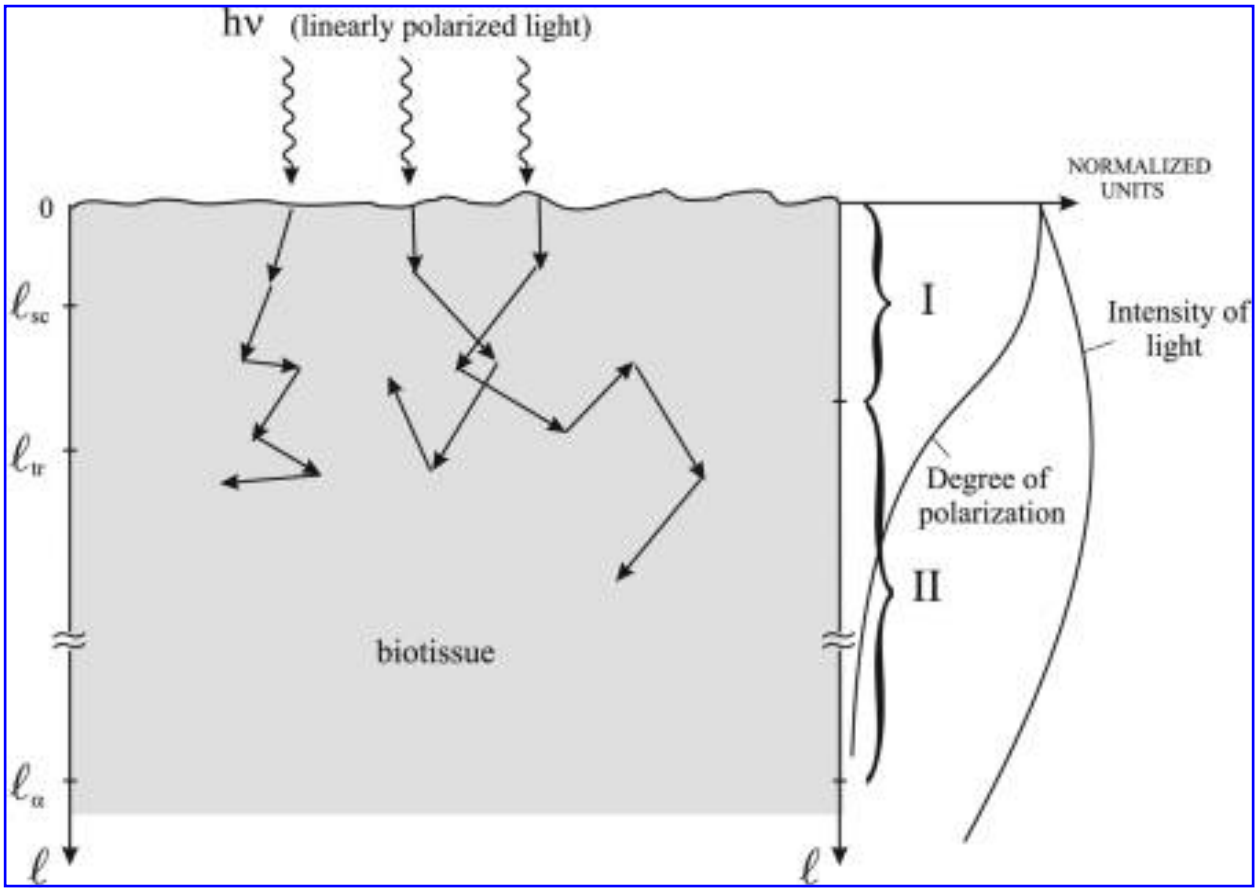

FIG. 2. A diagram of the effect of random scattering on light intensity and degree of polarization in scattering and absorbing ($\ell_a >> \ell_{sc}$) biotissue. ℓ = length of penetration; ℓ_a = length of light absorption; ℓ_{tr} = length of light transport; ℓ_{sc} = light scattering length. Polarization is maintained in zone I, and speckles exist in zone II, but in case of reflection by the rough surface, speckling may also occur in zone I.

20 cm^{-1} and $\mu_a \simeq 04$ cm^{-1} (μ_a is the absorption coefficient).[20] This means that it penetrates $\ell_{tr} \approx 0.5$ mm into skin tissue, and the light maintains its linear polarization until it reaches a depth of $\simeq 1.2$ mm. In deeper layers, the linear polarization is lost.

In our experimental conditions, depolarization of the light could not occur due to the small value of μ'_s L (i.e., due to the small probability of scattering, L is the depth of the irradiated layer, 3 mm; Fig. 1A). The results obtained (Tables 1 and 2) show that the photochemical effects of low-intensity light do not depend on light polarization in the cell suspension with isotropic orientation of the absorbers.

Our model system with distant and non-interacting cells is quite different from the highly organized composition of cells in a biotissue. In our suspension, the distance between the cells is on average $\simeq 100$ μm, which is not comparable with their diameter d $\simeq 20$–30 μm. This property sharply reduces the interaction of the cells during irradiation. In a biotissue, the interactions between cells during irradiation are not avoided *a priori*. This means that at least in principle, a biotissue may have sensitivity to light polarization. But in any case, at a depth of ≥ 2.5 ℓ_{tr}, any effect of polarization must disappear. However, two types of exceptions should be considered. First, so-called "ballistic" photons, able to penetrate to a greater depth, could allow polarization of deeper layers.[21] Second, some cell types (e.g., blood cells) can transport the irradiation's effects away from the irradiated zone. For these reasons, the question of the potential effects of polarization on irradiated biotissue cannot be resolved fully on the basis of model experiments with cell suspensions.

One should also consider the scattering interference (speckle) effect seen when a biotissue is irradiated. Let us consider two layers (zones) of a biotissue (Fig. 2, right side). At the top is the surface layer (I) with a depth of $\leq 2.5\ell_{tr}$, and beneath it lies a second layer (II), between $2.5\ell_{tr}$ and ℓ_a (here ℓ_a is the depth of absorption). $l_a = 1/\mu_a$. The border between the zones is poorly defined. In layer I, spatial nonhomogeneity of the laser beam is small, due to the small amount of scattering that occurs here, and the polarization may have a substantial effect. The polarization can cause, for example, the appearance of different gradients of absorbed light energy on the cell dimensions $d >> \lambda$.

In layer II, the light scattering is more substantial. The propagation of the laser light in a scattering biotissue has a so-called "speckle pattern." The speckle pattern is a random intensity distribution that is formed when fairly coherent (and polarized) laser beam propagates through a medium with random refractive index fluctuations.[22] This random intensity distribution appears with a spatial scale of about $\lambda/2$. Let us note that the contrast of the speckle intensity distribution depends on the degree of polarization of the beam.[22] However, back-scattering of the radiation can reflect from the rough skin surface, which may causes speckles to appear with diameter $\lambda/2$, even in layer I. This means that in both layers (I and II) of irradiated tissue, gradients of density of absorbed energy of varying degrees can occur. These may cause gradients of local heating in the biotissue (in the case of a pulsed beam, this is transient local heating[23]). These effects should be taken into account when considering the mechanisms of laser phototherapy at the biotissue level.[24]

CONCLUSION

Elementary processes in cells (light absorption, photochemistry, and biochemical reactions of photosignal transduction inside a cell) that occur during irradiation at $\lambda = 637$ nm, with a 30-min incubation period post-irradiation, do not depend on the degree of polarization of the light, as assessed using our cell suspension model. However, further experiments on actual biotissues are needed to acquire a fuller understanding of the possible role of light polarization in phototherapy.

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REFERENCES

1. Karu, T.I., Kalendo, G.S., Letokhov, V.S., and Lobko, V.V. (1982). Biostimulation of HeLa cells by low intensity visible light. Nuovo Cimento D 1, 828–840.
2. Bertoloni, G., Sacchetto, R., Baro, E., Ceccherelli, F., and Jori, G. (1993). Biochemical and morphological changes in *Escherichia coli* irradiated by coherent and noncoherent 632.8 nm light. J. Photochem. Photobiol. B. Biol. 18, 191–196.
3. Karu, T.I. (2003). Low power laser therapy, in: *Biomedical Photonics Handbook*. T. Vo-Dinh, (ed.). Boca Raton: CRC Press, pp. 48-1–48-25 (Chap. 48).
4. Karu, T.I. (2007). *Ten Lectures on Basic Science of Laser Phototherapy*. Grängesberg (Sweden): Prima Books AB, 2007.
5. Kubasova, T., Horvath, M., Kocsis, K., and Fenyö, M. (1995). Effect of visible light on some cellular and immune parameters. Immunol. Cell. Biol. 73, 239–244.
6. Zhevago, N.A., Samoilova, K.A., and Obolenskaya, K.D. (2004). The regulatory effect of polychromatic (visible and infrared) light on human humoral immunity. Photochem. Photobiol. Sci. 3, 102–108.
7. Bolton, P., Dyson, M., and Young, S. (1992). The effect of polarized light on the release of growth factors from the U-937 macrophage-like cell line. Laser. Ther. 4, 33–37.
8. Chumak, A.G., Chickan, D.N., and Ulaschik, V.S. (2000). Effect of polychromatic polarized light on afferent traffic in skin branches of somatic nerves. Bull. Exp. Biol. Med. 130, 624–626.
9. Ribeiro. M.S., Da Silva, D.F., De Aranjo, C.E., et al. (2004). Effects of low-intensity polarized visible laser radiation on skin burns: A light microscopy study. J. Clin. Laser. Med. Surg. 22, 59–66.
10. Satoh, N. (1997). Clinical application of linear polarized near-infrared (Super Lizer) therapy. Pain Clinic. 18, 1–9.
11. Yokoyama, K., and Oku, T. (1999). Rheumatoid arthritis–affected temporo-mandibular joint pain analgesia by linear polarized near infrared irradiation. Can. J. Anes. 46, 683–687.
12. Basford, J.R., Sandroni, P., Low, P.A., Hines, S.M., Gehrking, J.A., and Gehrking, T.L. (2003). Effects of linearly polarized 0.6-1.6 μm irradiation on stellate ganglion function in normal subjects and people with complex regional pain (CRPSI). Lasers Surg. Med. 32, 417–423.
13. Yokoyama, K., and Sugiyama, K. (2003). Influence of linearly polarized near-infrared irradiation on deformability of human stored erythrocytes. J. Clin. Laser Med. Surg. 21, 19–22.

14. Monstrey, S., Hoeksema, H., Saelens, H., et al. (2002). A conservative approach for dermal burn wounds using polarized-light therapy. Br. J. Plastic Surg. 55, 420–426.
15. Pinheiro, A.L., Pozza, D.H., Oliveira, M.G., Weissmann, R., and Ramalho, L.M. (2005). Polarized light (400-2000 nm) and non-ablative laser (685 nm): A description of the wound healing process using immunohistochemical analysis. Photomed. Laser Surg. 23, 485–492.
16. Kubasova, T., Fenyö, M., Somoszy, Z., Gazso, L., and Kertesz, I. (1988). Investigations on biological effect of polarized light. Photochem. Photobiol. 48, 505–509.
17. Karu, TI.,. Pyatibrat, L.V., Kalendo, G.S., and Esenaliev, R.O. (1996). Effects of monochromatic low-intensity light and laser irradiation on adhesion of HeLa cells *in vitro*. Lasers Surg. Med. 18, 171–177.
18. Karu, T. (1999). Primary and secondary mechanisms of action of visible-to-near radiation on cells. J. Photochem. Photobiol. B Biol. 49, 1–17.
19. Jacques, S.L., Ostermeyer, M.R., Wang, L., and Down, S.V. (1996). Polarized light transmission through skin using video reflectometry: Toward optical tomography of superficial tissue layers. Proc. SPIE 2671, 199–210.
20. Tuchin, V.V. (1997). Light scattering study of tissues. Physics-Uspekhi. 167, 517–539.
21. Demos, S.G., Savage, H., Heerdt, A.S., Schantz, S., and Alfano, R.R. (1996). Time resolved degree polarization for human breast tissue. Optics Comm. 124, 439–442.
22. Goodman, J.W. (1984). Statistical properties of laser speckle pattern, in: *Laser Speckle and Related Phenomena*. J.C. Dainty, (ed.). Berlin: Springer-Verlag.
23. Letokhov, V.S. (1991). Effects of transient local heating of spatially and spectrally heterogeneous biotissue by short laser pulses. Nuovo Cimento. D 13, 939–948.
24. Letokhov, V.S. (2003). Laser light in biomedicine and the life sciences from the present to the future, in: *Biomedical Photonics Handbook*. T. Vo-Dinh, (ed.). Boca Raton: CRC Press, pp. 5–16.

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