Chapter IV

CELLULAR MECHANISMS OF LOW POWER LASER THERAPY: NEW QUESTIONS

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1. Introduction

Low power laser therapy (photobiomodulation, laser biostimulation) was advanced more than 30 years ago. Since then medical treatment with coherent light sources (lasers) or noncoherent light (Light Emitting Diodes, LED's) has passed through its childhood and early maturity. The controversial points of laser biostimulation, which were topical at the end of 80ties (reviews [1-3]), are not topical any more. There is no doubt nowadays that low-intensity monochromatic light from lasers of LED's acts directly on the organism at the molecular level. The photoacceptors, primary physical and chemical reactions in/with photoacceptors, and possible cellular signaling have been already investigated in some extent (reviews [4-7]. It is believed that there excists an universal photobiological mechanism of light action on respiratory chain in both eukaryotic and prokaryotic cells (terminal enzymes of the respiratory chain being the photoacceptors). The specificity of cellular responses appear only during secondary reactions (cellular signaling). The primary and secondary mechanisms on cellular level were also summarized in the first book of the present trilogy [8].

Beside the activation of cell metabolism through respiratory chain, there are also other additional ways of light activation of differentiated cells (e.g., phagocyting cells) [6]. Last, but not least, interactions between various cell types on tissue level exist, this circumstance making the mechanisms of low-power laser therapy much more complicated. We are still far away from the full understanding of low-power laser radiation action mechanisms on tissue and organism levels.

In the present contribution, three new recently arisen problems are analyzed. One of the most topical points of low-power laser medicine today is the following: has coherent and polarized light additional benefits in comparison with noncoherent light at the same wavelength and intensity. This problem is considered in Section 2.

It is generally believed that low-power laser therapy has no hazards on patents health. This is true for short-term time scale. No investigations have been performed in long-term time scale. This

question on cellular level is discussed in Section 3.

Section 4 considers possible protective and preventive effects of monochromatic visible-to near IR radiation on cellular level.

2. Has coherent and polarized light additional benefits in comparison with noncoherent light at the same wavelength and intensity?

Clinical applications of low power laser therapy are diverse. This field is characterized by a variety of methodologies and uses of various light sources (lasers, LEDs) with different parameters (wavelength, output power, continuous wave or pulsed operation modes, pulse parameters). Fig.1 presents schematically the types of light therapeutic devices, possible wavelengths they can emit, and maximal output power used in therapy.

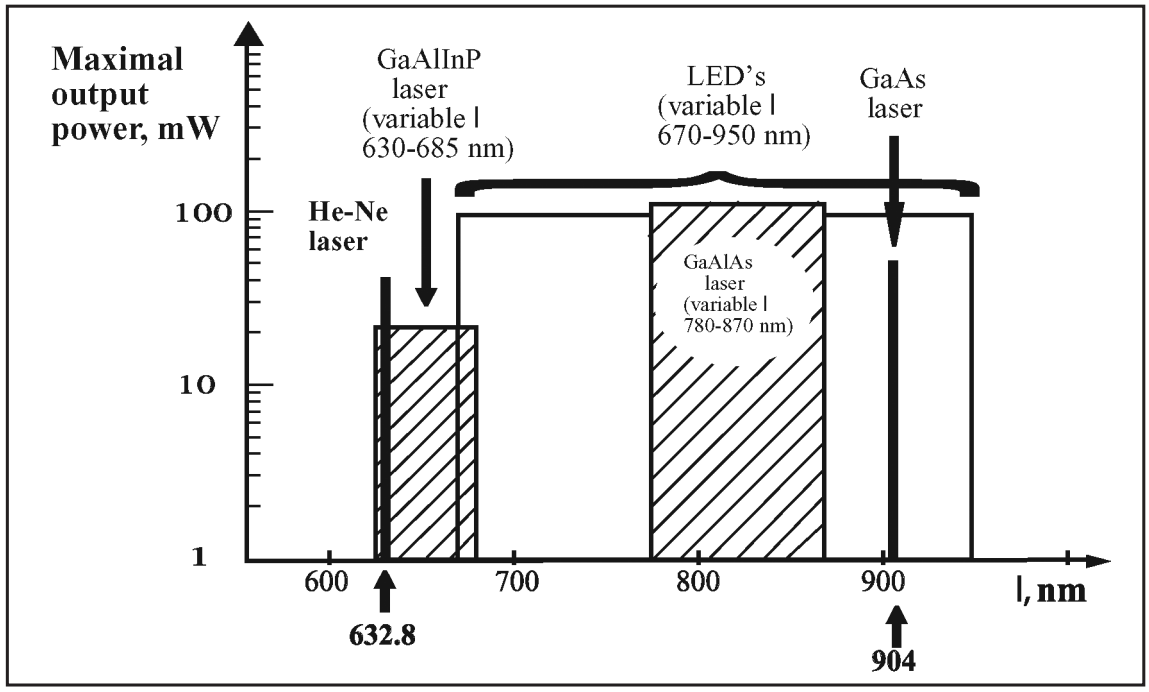

Fig.1. Wavelength and maximal output power of lasers and LED's used in low power laser therapy.

The GaAlAs diodes are used both in diode lasers and LED's, the difference is whether the device contains the resonator (as the laser does) or not (LED). The wavelengths (~620-900 nm) used and output powers (to 100 mW) are practically the same in both types of therapeutic devices. In this connection, one of the most topical and widely discussed points in the low-power laser therapy clinical community is the following: can the coherence and polarization of laser radiation have some additional benefits as compared with monochromatic light from a conventional light source or LED with the same wavelength and intensity.

It is necessary to distinguish two aspects of this problem: the *coherence of light* itself and the *coherence of the interaction* of light with matter (biomolecules, tissues).

A). First, let us to consider the *coherence of the light*. The coherent properties of the light are described by *temporal* and *spatial* coherence. Temporal coherence of the light is determined by the spectral width $\Delta\nu$, since the coherence time τ_{coh} during which light oscillations in the point of irradiation occur, has a regular and strongly periodical character:

$$\tau_{coh} \cong \frac{1}{\Delta\nu} \qquad (1).$$

Here $\Delta\nu$ is the spectral width of the beam in Hz. Since the light propagates with the speed $c=3 \times 10^{10}$ cm/s, the light oscillations are matched by the phase (i.e., they are coherent), on the length of light propagation L_{coh}

$$L_{coh} \cong \frac{C}{\Delta\nu[Hz]} \qquad \text{or,} \qquad L_{coh} \cong \frac{C}{\Delta\nu[cm^{-1}]} \qquad (2).$$

L_{coh} is called longitudinal coherence. The more monochromatic is the light, the longer is the length where the light field is coherent in volume. For example, for a multimode He-Ne laser with $\Delta\nu$ = 500 MHz, L_{coh}= 60 cm. But for a LED emitting at

λ=800 nm (=12500 cm^{-1}), $\Delta\nu$=160 cm-1 (or $\Delta\lambda$ =10 nm) and L_{coh}=1/160cm^{-1} ≅ 60 μm, i.e. L_{coh} is longer than the thickness of a cell monolayer (≈10-30 μm).

Spatial coherence describes a correlation between the phases of the light field in a lateral direction. For this reason, spatial coherence is also called lateral coherence. The size of the lateral coherence (ℓ_{coh}) is connected with the divergence (φ) of the light beam at the point of irradiation:

$$\ell_{coh} \cong \frac{\lambda}{\varphi} \qquad (3).$$

For example, for a He-Ne laser, which operates in the TEM_{00} mode, the divergence of the beam is determined by the diffraction

$$\varphi \cong \frac{\lambda}{D} \qquad (4).$$

where D is the beam diameter. In this case, ℓ_{coh} coincides with the beam diameter, since for the TEM_{00} laser mode the phase of the field along the wave front is constant.

With conventional light sources, the size of the emitting area is significantly larger than the light wavelength and various parts of this area emit light independently or noncoherently. In this case, the size of the lateral coherence ℓ_{coh} is significantly less than the diameter of the light beam and ℓ_{coh} is determined by the light divergence as seen in the formula (3).

An analysis of published clinical results from the point of view of different types of radiation sources does not allow one to conclude that lasers have a higher therapeutic potential than LED's. But in some certain clinical cases the therapeutic effect of coherent light is believed to be higher [9]. However, when human peptic ulcers were irradiated by a He-Ne laser or properly filtered red light in a specially designed clinical double blind study, equally positive results were documented for both types of radiation sources ([10], review [3]).

B). Let us consider now the *coherence of light interaction* with biomolecules, cells, and tissues. The coherent properties of light are not manifested when the beam interacts with a biotissue on the molecular level. This problem was first considered years ago (review [1]). Then the question was whether coherent light is needed for "laser biostimulation" or is this simply a photobiological phenomenon. The conclusion was that under physiological conditions, the absorption of low-intensity light by biological systems is of purely noncoherent (i.e., photobiological) nature because the rate of decoherence of excitation is many orders of magnitude higher than the rate of photoexcitation. The time of decoherence of photoexcitation determines the interaction with surrounding molecules (at normal conditions less than 10^{-12} s). The average excitation time depends on the light intensity (at

an intensity of 1mW/cm^2 this time is around 1s). At 300 K in condensed matter for compounds absorbing monochromatic visible light, the light intensity at which the coherent light-matter interactions start to occur was estimated to be above the GW/cm^2 level [1]. Note that the light intensities used in clinical practice range are not higher than tens or hundreds of mW/cm^2. Indeed, the stimulative action of various bands of visible light at the level of organisms and cells was known long before the advent of the laser. Also, specially designed experiments at the cellular level have provided evidence that coherent and noncoherent light with the same wavelength, intensity, and irradiation time provide the same biological effect [11-13]. Successful use of LED's in many areas of clinical practice also confirms this conclusion.

Therefore, it is possible that the effects of light coherence are manifested on the macroscopical (e.g., tissue) level, at various depths (L) of irradiated matter. In Fig. 2, the coherence volumes (V_{coh}) and coherence lengths (L_{coh}) for four different light sources are presented. Fig.2A presents the data for two coherent light sources (He-Ne and diode laser as typical examples of therapeutic devices). Fig.2B presents the respective data for noncoherent light (LED and spectrally filtered light from a lamp). It is seen in Fig.2 that large volumes of tissue are irradiated only by laser sources with monochromatic radiation (Fig.2A). For noncoherent radiation sources (Fig.2B), the length of the coherence L_{coh} is small. It means that only surface layers of irradiated substance can be achieved by coherent light.

The spatial (lateral) coherence of the light source is not important due to strong scattering of light in biotissue when propagated to the depth $L >> \ell_{SC}$, where ℓ_{SC} is the free pathway of light in relation to scattering. The reason is that every region in a scattering medium is illuminated by radiation with a wide angle ($\varphi \approx 1$rad). This means that $\ell_{coh} \cong \lambda$, i.e. the size of spatial coherence ℓ_{coh} decreases to the light wavelength (Fig.2).

So, the length of longitudinal coherence (L_{coh}) is important when bulk tissue is irradiated, since this parameter determines the volume of the irradiated tissue V_{coh}. In this volume, the random interference of scattered light waves and formation of random non-homogeneities of intensity in space

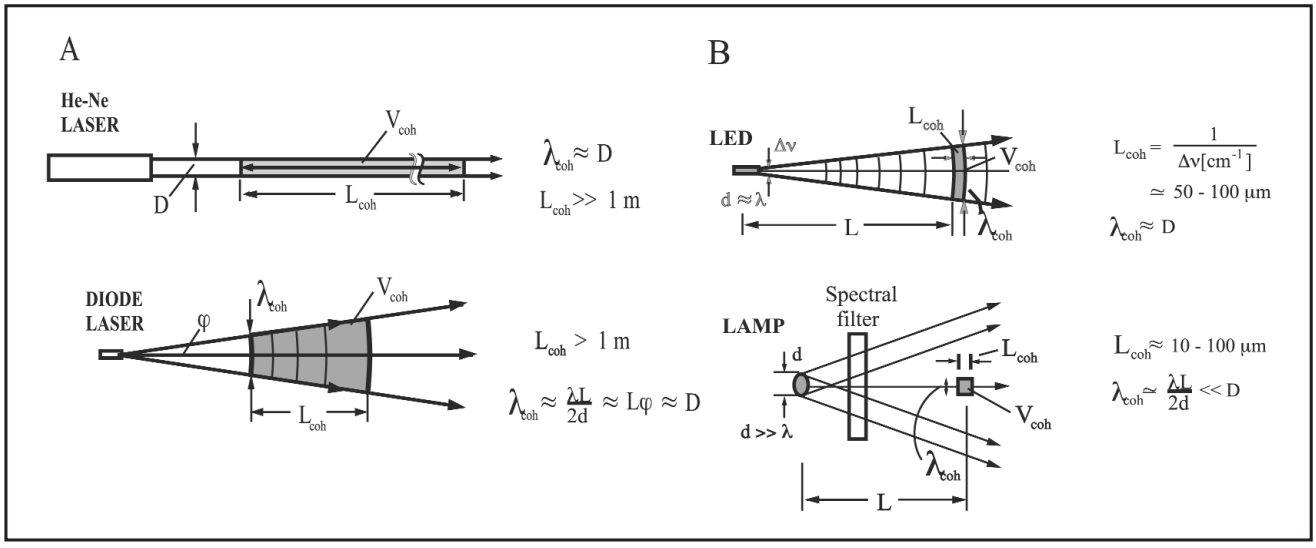

Fig. 2. Coherence volumes and coherence lengths of light from: (A) laser and (B) conventional sources when a tissue is irradiated. L_{coh} - length of temporal (longitudinal) coherence, ℓ_{coh} - size of spatial (lateral) coherence, D - diameter of light beam, d - diameter of a noncoherent light source, φ - divergence of the beam, Δν - spectral width of the beam.

(speckles) occur. For noncoherent light sources, the coherence length is small (tens to hundreds of microns). For laser sources, this parameter is much higher. So, the additional therapeutic effect of coherent radiation, if this exists indeed, depends not only on the length of L_{coh}, but mainly on the penetration depth into the tissue due to absorption and scattering, i.e. by the depth of attenuation. Table 1 summarizes qualitative characteristics of coherence of various light sources as discussed above.

Light source	**Qualitative characteritics of coherence**			
	Temporal coherence	Length of longitudinal (temporal) coherence, L_{coh}	Spatial coherence	Volume of spatial (lateral) coherence, ℓ_{coh}
Laser	very high	very long	very high	large
LED	low	short (>>λ)	high	small (very thin layer)
Lamp with a spectral filter	low	short (>>λ)	very low	very small
Lamp	very low	very short (≈λ)	very low (≈λ)	extremely small (≈λ^3)

Table 1. Comparison of coherence (temporal and spatial) of various light sources used in clinical practice and experimental work

Difference in the coherence length L_{coh} is not important when thin layers are irradiated inasmuch as the longitudinal size of irradiated object $\Delta\ell$ is less than L_{coh} for any source of monochromatic light (filtered lamp light, LED, laser). Examples are the monolayer of cells and optically thin layers of cell suspensions (Fig.3 A, B). Indeed, experimental results [8-10] on these models clearly provide evidence that the biological responses of coherent and noncoherent light with the same parameters are equal. The situation is quite different when a bulk tissue is irradiated (Fig.3C). The coherence length L_{coh} is very short for noncoherent light sources and can play some role only on surface layers of the tissue with thickness $\Delta\ell_{surface}$. For coherent light sources, the coherence of the radiation is kept along all penetration depth L. On all this distance in bulk tissue ($\Delta\ell_{bulk}$), the random interference of light waves of various directions occurs. As a result, a speckle pattern of intensity appears. Maximums of the intensity appear at the random constructive interference. At the random destructive interference, the minima (i.e., regions of zero intensity) occur. The dimensions of these speckles at all directed random interference are approximately in range of the light wavelength λ. The coherent effects (speckles) appear only in the depth L_{coh}. These laser- specific speckles cause a spatially nonhomogeneous deposition of light energy and respectively, they lead to statistically nonhomogeneous photochemical processes, an increase of temperature, changes in local pressure, deformation of cellular membranes etc.

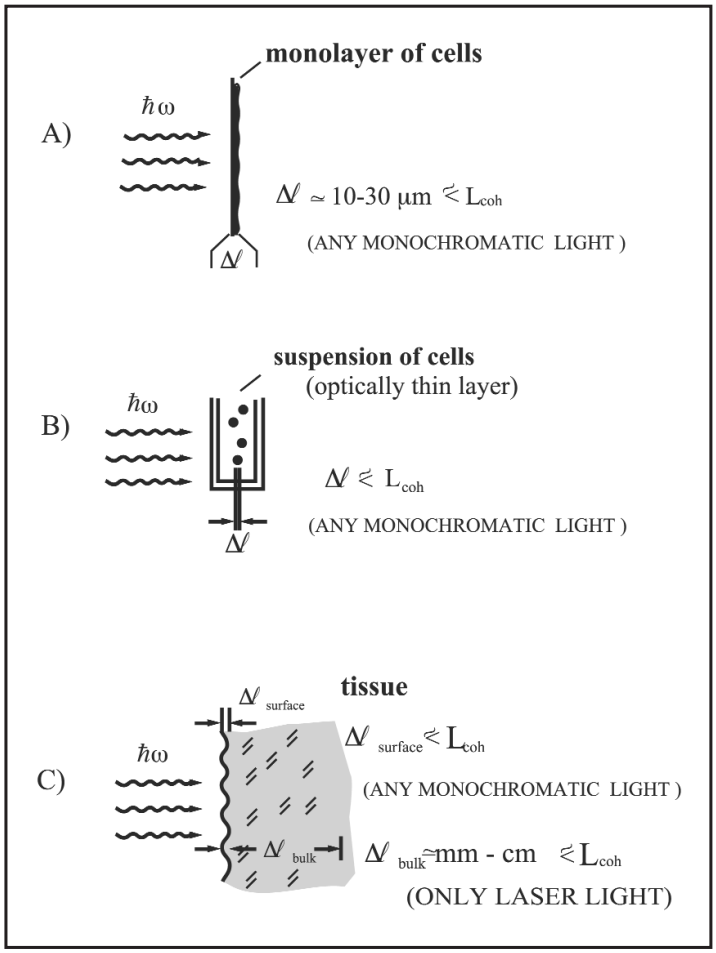

Fig.3. Depth (Δl) in which the beam coherency is manifested and coherence length L_{coh} in various irradiated systems: (A) monolayer of cells, (B) optically thin suspension of cells, (C) surface layer of tissue and bulk tissue.

For nonpolarized coherent light, the random speckles are less pronounced (they have lower contrast) as compared to the speckles caused by coherent polarized light. A special feature of nonpolarized coherent radiation is that the regions with zero intensity appear less often as compared with the action of coherent polarized light. Thus, the polarization of light causes brighter random intensity gradients that can enhance the manifestation of the effects of light coherence when the tissue is irradiated.

Thus, it is possible to conclude that in scattering biotissue the main role is played by the coherence length (monochromaticity of light), inasmuch as this parameter determines the depth of tissue where the coherent properties of light beam can potentially manifested on dependence of the attenuation. This is the spatial (lateral) coherence of the beam, i.e. its directivity, which plays the main role in delivery of light into biotissue. One has to add that the direction and orientation of laser radiation could be important factors for some types of tissues (e.g., dental tissue) which have fiber-type structures (filaments). In this case wave-guide propagation effects of light can appear, which provide an enhancement of penetration depth.

Taking together in the framework of this qualitative picture, some possible additional (i.e., additional to those effects caused by light absorption by photoacceptor molecules) manifestation of light coherence for deeper tissue is quite possible. This qualitative picture explains also why coherent and noncoherent light with the same parameters provide the same biological effects on cell monolayer [11], thin layers of cell suspension [12,13], and tissue surface (e.g., by healing of peptic ulcers [10]). Some additional (therapeutical) effects from the coherent and polarized radiation can appear only in deeper layers of the bulk tissue. By now, no experimental work has performed to

study these possible additional effects qualitatively and quantitatively. In any case the main therapeutical effects occur due to light absorption by cellular photoacceptors.

3. Can irradiation with monochromatic light of visible and near IR spectral region cause long-term effects, which appear in following cell generations?

Experimental data considering possible hazardous (e. g., mutagenic) effects of low power laser radiation is not numerous. It is know that the irradiation with a He-Ne laser caused an increase in frequency of chromosome abberations in diploid cells of human fibroblasts [14] and irradiation with a semiconductor laser at 660 nm increased output of single-strand breaks of DNA in dose-dependent manner [15].

The radiation with $\lambda = 632.8$ nm or 660 nm used in experiments [14,15] cannot cause mutations through direct action upon DNA. The energy of these photons is too low (~1.7 eV) to cause ruptures of covalent bonds in a molecule. DNA and RNA also do not have absorption bands in the visible spectral region. So, the results of works [14,15] cannot be explained by a direct action of visible light on DNA, and one has to suppose indirect effects.

On the other side, the proliferation of mammalian cell cultures (review [16]) as well as the division of yeasts were increased during many generations after a short-time irradiation [17-19]. These data support the suggestion that some genetic (mutagenic?) effects can be involved.

We studied the long-term effects of He-Ne radiation on the yeast culture *Torulopsis sphaerica* [20-23]. The main goal of these studies was to investigate whether the functional activation of mitochondria of initially irradiated cells is accompanied by ultrastructural changes in mitochondria in successive generations. Earlier, it was found that the activity of some respiratory chain enzymes was still elevated in the cells of successive generations of the irradiated cells [19].

In the experiments described in [20-23], two doses, 460 J/m^2 and 1150 J/m^2, marked as points A and B on the dose-dependence curve of protein synthesis stimulation in Fig. 4, were used. These doses were chosen because at the point A the protein synthesis and enzyme activites were maximally elevated above control level and at the point B, these parameters were again close to the control level (Fig. 4). The cells were irradiated with He-Ne laser in buffer where the yeasts are not dividing and then cultivated in the nutrient medium for 18 h. During this time of cultivation the cells went through 6-7 generations.

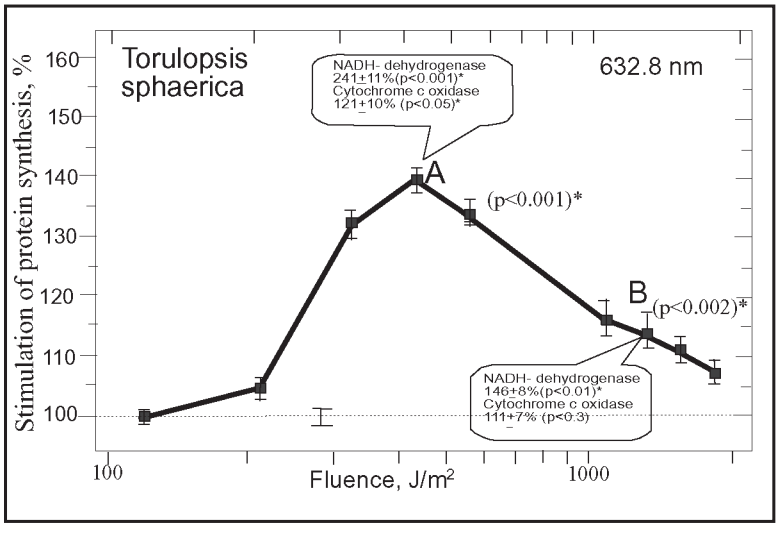

Fig. 4. Amount of synthesized protein as a percentage of control level measured 18h after the irradiation of yeast in various doses in buffer and seeding in the nutrial medium (adapted from [18]). Points A and B denote the doses chosen for the electron microscopy in the present study. Respective values of activity of NADH-dehydrogenase and cytochrome c oxidase are from the paper [19]. Asterisks denote statistically significant results.

In our study we found changes in the ultrastructure of the chondriome of yeast cells, which ancestors were irradiated with a He-Ne laser. In both irradiated groups, i. e. at the dose 460 J/m^2 and 1150 J/m^2, the ultrastructure of mitochondria was found to be different from that of the control cells. The chondriome of the cells, which ancestors were irradiated at the dose 460 J/m^2 was found to be different from that of control cells only quantitatively (Table 2, Figs. 5,6). In case of the irradiation at the dose 1150 J/m^2, the mitochondrial apparatus of cells-descendants differed from that of control cells both quantitatively (Table 2, Fig. 5) and qualitatively (Fig. 6). Spatial reconstruction of the mitochondrial apparatus of intact budding cells established presence of one branched giant mitochondrion and in average three mitochondria organized in the reticulum. As seen in Fig. 6A, a part of the giant mitochondrion is displaced into bud.

Table 2. Quantitative characteristics of mitochondrial profiles in cell section (M±SEM)

Experimental group	Number of mitochondria per cell section	Number of mitochondria per μm^2 of cytoplasm	Area of a mitochondrion, μm^2	Area of chondriome per area of cytoplasm, μm^2/μm^2	Area of cristae per area of mitochondrion, μm^2/μm^2	Per cent of small (S≤0.06 μm^2) mitochondria	Per cent of large (S≥0.3μm^2) mitochondria
Control	3.8 ± 0.3	0.22 ± 0.02	0.17 ± 0.02	0.037 ±0.005	0.20 ± 0.01	41	12
Cells which ancestors were irradiated at 460 J/m^2	3.5 ± 0.3	0.17 ± 0.02 p< 0.05	0.26 ± 0.02 p< 0.001	0.045 ±0.006	0.25 ± 0.01 p< 0.001	22 p<0.05	28 p< 0.01
Cells which ancestors were irradiated at 1150 J/m^2	4.8 ± 0.3 p< 0.05 p'<0.01	0.28 ± 0.02 p< 0.05 p'<0.001	0.14 ± 0.01 p'<0.001	0.039 ± 0.004	0.19 ± 0.01 p¢<0.001	55 p<0.05 p'<0.001	7 p'<0.002

p - significance level between experimental group and control
p' - significance level between two experimental groups.

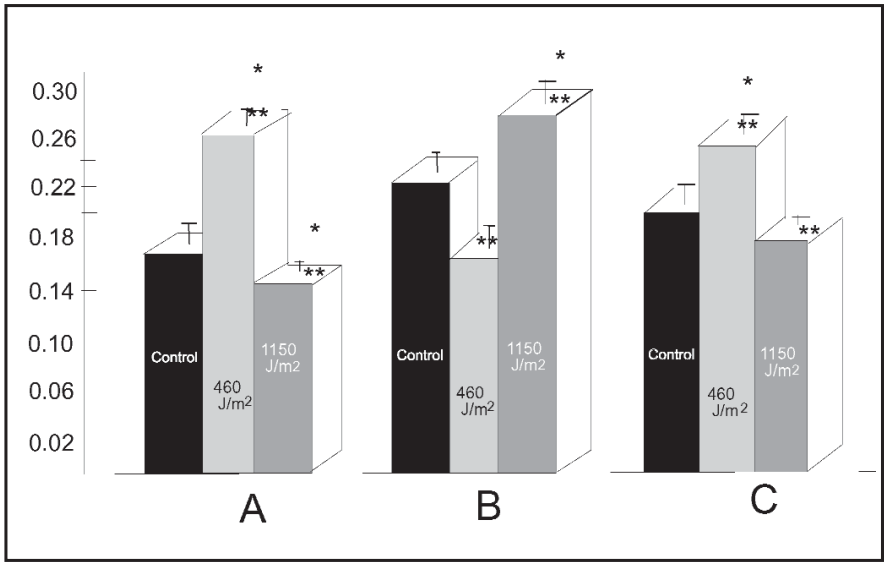

Fig. 5. Changes in parameters of mitochondria: A - average area of a mitochondrion in μm^2, B - number of mitochondria per μm^2 of cell cytoplasm, C - relation of the area occupied by cristae to the area of a mitochondrion (μm^2/μm^2) (adapted from [22]).
*p<0.001 (between control and irradiated group).
**p<0.05 (between two irradiated group).

The reconstruction of the chondriome of the budding cells, which ancestors were irradiated at 460 J/m^2 evidenced the presence of one giant mitochondrion. The giant mitochondrion was distributed between the mother cell and the bud (Fig. 6B). The chondriome also involved ordinary small organelles.

Cells which ancestors were irradiated in dose 1150 J/m^2, were characterized by changing of spatial organization of the chondriome as compared with both control cells and the cells from the another irradiated group. A great number of small organelles was revealed in the budding yeast cells (Fig. 6C). Giant mitochondria were not found in this experimental group.

The presence of giant mitochondria in yeast cells is not a unique phenomenon. For example, this type of mitochondrial apparatus was found in yeasts during their switching from aerobic respiration to glycolysis [24], or in cultures grown with limited glucose [25]. At the same time, the giant mitochondria were also present in exponentially growing yeasts with active respiration [26]. All yeast cultures in our experiments were in the same (exponential) phase of growth and were also cultivated in the same conditions in presence of glucose and oxygen. So, one can not connect changes in chondriome found in our experiments with variations in life cycle of yeasts and cultivation conditions.

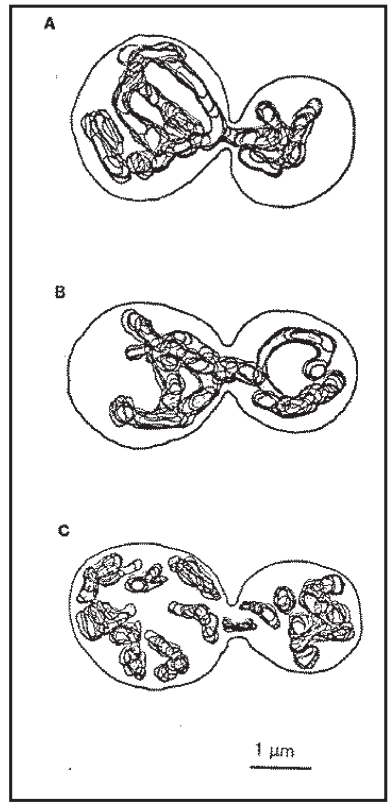

Fig. 6. Spatial reconstruction of chondriome from (A) control cells and cells which ancestors were irradiated at: (B) 460 J/m^2 or (C) 1150 J/m^2 (adapted from [21,22]).

One can find an accordance between the morphological changes of chondriome in irradiated cells (Table 2, Fig. 5) and activity of respiratory enzymes in the same cultures (Fig. 4). The mitochondria of cells, which ancestors were irradiated at 460 J/m^2 were characterized by increased area of cristae (Table 2) and increased activity of NADH-dehydrogenase and cytochrome c oxidase (Fig. 4). A correlation between increase in the area of mitochondrial cristae and activity of respiratory enzymes [27-29] as well as between decrease of area of cristae and inhibition of respiration [30] and decrease of transmembrance potential [31] has been described. A fragmentation of mitochondria per se was found by treating the cells with inhibitors of respiratory chain [32,33]. The area of cristae per area of a mitochondrion was decreased back to the control level in cells of the culture initially irradiated at 1150 J/m^2 (Table 2). Also, the activity of the respiratory enzymes was decreased as compared with respective data for the cells initially irradiated at 460 J/m^2 (Fig. 4). It is known that a decrease in the area of the cristae and an increase in the mitochondrial number per cell section were correlated with a decrease of respiration and oxidative phosphorylation [34,35].

Spatial reconstruction of the chondriome evidenced that in two cases (control group and the culture initially irradiated at 460 J/m^2) the giant mitochondria were present but they were absent in the culture intially irradiated at 1150 J/m^2 (Fig. 6). It must be recalled that the spatial reconstruction was carried out in all cases in budding cells after nucleokinesis when the cytokinesis was not finished. It means that the established changes in the structure of chondriome were not connected with cells at different points of the cell cycle. It is known that during the cell cycle mitochondria are fragmented to discrete organelles or infused into giant structures [36,37].

So, both morphometric and spatial analyses of the chondriome of cells-descentants of irradiat-

ed cultures testified that there are changes in the ultrastructure of the mitochondrial apparatus. These changes depended on the irradiation dose and correlated with functional activity of the respiratory chain. Since the experiments were performed with cells of 6^{th}-7^{th} generations of initially irradiated cultures, one can suppose that these changes are of genetic origin.

It is known that DNA is sensitive to oxidative damage and reactive oxygen species (ROS) can cause mutations particularly in mitochondrial DNA rather easily (review [38]). It was proposed that increased production of ROS like superoxide anion (and the product of the dismutation, H_2O_2) as a result of direct light activation of respiratory enzymes can be considered among primary mechanisms when monochromatic visible light affects mitochondria (reviews [4,5]). It means that one cannot exclude a possibility of mutational action of ROS in irradiated cells. The result of this action can appear in the cells-descendants.

Mitochondrial ATP synthesis (oxidative phosphorylation) is regulated by the membrane potential (respiratory control) and protein synthesis (transcriptional control) (reviews [39,40]). The first type of regulation can occur and really occurs only in directly irradiated cells reviews [5,6, 41]), so in the present case one can think mainly about possible transcriptional control. The following experimental finding supports the suggestion about involvement of transcriptional control: both mitochondrial and nuclear DNA encode four enzyme complexes of oxidative phosphorylation. These are F_0F_1, complex I, complex III and complex IV [39]. Recall here that in our experiments the activity of NADH-dehydrogenase and cytochrome c oxidase ([19], Fig. 4) belonging respectively to complexes I and IV, was increased.

We found that the total area of chondriome per area of cytoplasm was practically not changed due to the irradiation (Table 2). This fact could point to the absence of the activation of replication of mitochondrial DNA (mtDNA) [27,42]. It means that the changes in the ultrastructure of the inner mitochondrial membranes (cristae) (Table 2) correlating with the changes in the activity of two enzymes of respiratory chain [19] could be due to some changes in the transcription and/or translation functions of mtDNA.

The results of the papers [20-23] demonstrate that cells-descendants of the initially irradiated with a He-Ne laser culture have quantitative or qualitative (depending of the dose) changes in the mitochondrial ultrastructure. These results give grounds to suppose that the irradiation with He-Ne laser causes not only rapid regulation of ATP synthesis in directly irradiated cells [5,6, 41], but also can affect the control of mitochondrial activity via protein synthesis (transcription and/or translation control).

4. Can irradiation with monochromatic light of visible and near IR spectral region prevent or eliminate hazardous effects of chemicals?

Monochromatic visible-to-near-IR (laser) radiation stimulates various metabolic responses of cells (review: [6]) and modifies cell responses to ionizing [14, 43-48] and UV [49-51] radiation. Surprisingly enough, it was found that namely the pre-irradiation of cells with a monochromatic red light (He-Ne laser emitting at 632.8 nm) reduces their cytotoxic response to ionizing radiation (γ- and x- radiation, α- particle flow) [43-46]. This radioprotective effect depends on the He-Ne laser radiation dose, as well as on the time interval between the two irradiation events [44]. The mechanism of this phenomenon has not as yet been established, but it has been suggested that it is closely associated with the mechanism whereby cellular metabolism is stimulated by monochromatic visible-to-near IR radiation [6]. Later, it has been found that also other wavelengths of visible-to-near-IR optical region act radioprotectively [47,48] as well as induce protection against UV cytotoxicity

[51].

The adhesion of cells to extracellular matrices is the initial event of their growth *in vitro*. The adhesion of HeLa cells can be increased by their irradiation with low-intensity monochromatic visible light. This phenomenon has a well-structured action spectrum, which indicates that there exists a photoacceptor for it ([52], upper corner in Fig. 7).

It is known that many chemicals that affect certain metabolic pathways in cells inhibit their

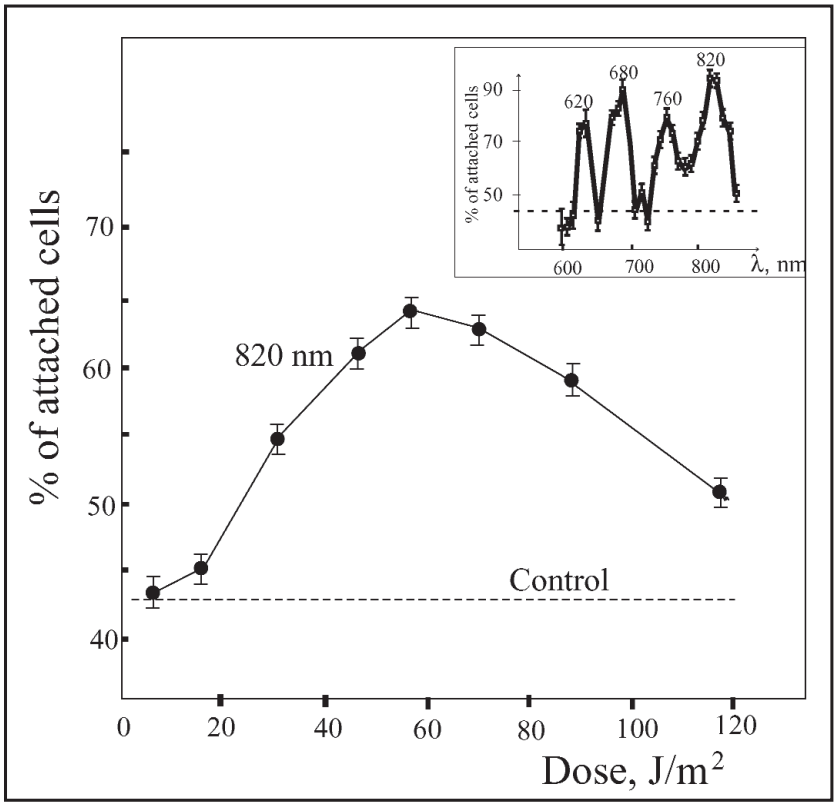

Fig. 7. Dose dependence of the cell attachment increase due to irradiation at 820 nm (adapted from [53]). The dashed line indicates the attachment of the control cells. Shown in the top right-hand corner is the dependence of the HeLa cell attachment on the wavelengths of continuous-wave red and near-IR radiation (D = 60 J/m^2) (modified from the paper [52]).

adhesion as well. The aim of our experiments [53-56] was to investigate whether the irradiation of a suspension of HeLa cells at λ = 820 nm prior to or after their being treated with certain chemicals can modify the effect of the latter on the adhesion of the cells to a glass matrix. The chemicals tested included antioxidants (free-radical scavengers), respiratory-chain inhibitors, NO donors, chemicals inhibiting the phospholipase A_2 or monovalent-ion flows through the plasma membrane, and thiol-reactive compounds.

In our experimental conditions, 42.5±2.5% of the total number of cells in a cuvette (85 000) attach themselves to the bottom of the vial. Irradiation increases the number of the cells attached to the glass in a wavelength- as well as dose-dependent manner. The dose dependence of cell adhesion is presented in Fig. 7. This curve is bell-shaped, with a maximum at 60 J/m^2; the percentage of the adhered cells at this point amounting 64.5±3.1%. In subsequent experiments, the radiation dose was 60 J/m^2.

Shown in the top right-hand corner of Figure 7 is the relationship between the adhesion of HeLa cells and the wavelength of light used for irradiation (the so-called action spectrum which resembles the absorption spectrum of the primary photoacceptor). This spectrum was recorded using the same cell adhesion assessment method [52]. There are two reasons for presenting the action spectrum here. First, it explains our choice of the radiation wavelength (820 nm) in the present experiments:

one of the maxima in the action spectrum is at 820 nm. Secondly, it can be seen from Fig. 7 that the effect of light on such an integral parameter as cell adhesion is represented by a well-structured action spectrum. And what is more, this action spectrum is practically the same as recorded for DNA and RNA synthesis rate [1-3] i.e., for the processes occurring in the nucleus. This is the evidence that the same photoacceptor absorbing visible-to-near-IR radiation is involved.

Most of the chemicals under study inhibit the attachment of HeLa cells to glass (Table 3, column 2). One can see from Table 3 that fifteen chemicals (items 1 through 15) inhibit cell attachment statistically significantly, two more (items 16 and 17) stimulate cell attachment, while another six (items 18 through 23) have no effect at all as compared with the controls.

Column 3 in Table 3 presents the percentage of cells adhered to glass in the case where the suspension was irradiated immediately before the addition of a chemical. In this series of experiments, the radiation dose amounted to 60 J/m^2 (at this dose the increase of cell attachment reaches its maximum, Fig. 7). Comparison between the data listed in columns 2 and 3 of Table 3 shows that the attached cell percentage is higher in pre-irradiated samples in most cases. As shown by the analysis below, there are four possibilities.

First, pre-irradiation reduces the inhibitive effect of the chemical, but the attached cell percentage is still lower than that in the controls. Such chemicals include GSH (item 14 in Table 3), H_2O_2 (item 15), melatonin (item 12), quinacrine (item 11), DNP (item 10), ATP (item 8), and ouabain (item 3). Second, the inhibitive action of the chemical is fully eliminated by pre-irradiation. In the case of rotenone (item 9), the attached cell percentage (43.5±3.3 %) is close to that in the control (42.5±2.5 %). Third, the attached cell percentage in the pre-irradiated sample is higher than in the control sample (42.5±2.5 %). This is true of arachidonic acid (item 1), SOD (item 2), catalase (item 4), SOD (item 5), ouabain (item 6), azide (item 7), mannitol (item 13), GSSG (item 18), and $NaNO_2$ (item 19). Fourth, chemicals increase the attachment of cells and the attached cell percentage in the pre-irradiated sample is even higher. This is true for amiloride (item 16) and methylene blue (item 17 in Table 3).

However, comparison between the data listed in columns 2 and 3 of Table 3 shows that there are four chemicals for which the attached cell percentage in pre-irradiated samples is very close to that in their nonirradiated counterparts. These chemicals are SNP (item 20 in Table 3), 2-mercaptoethanol (item 21), cysteine (item 22), and $CuSO_4$ (item 23). This means that pre-irradiation with these chemicals has no effect on cell attachment.

The linear regression analysis of the data listed in columns 2 and 3 of Table 3 points to the existence of correlation between them. The correlation coefficient is equal to 0.4332, which is statistically significant ($P = 0.0006$). This analysis has involved all the 23 pairs of samples. When subject to the analysis are only those pairs of samples whose pre-irradiation increases the attached cell percentage (items 1 through 19 in Table 3), the correlation coefficient proves higher, namely, 0.6207 ($P < 0.0001$) (Figure 8).

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Table 3. Modification of cell attachment with radiation (60 J/m^2) and chemicals

Chemical	% of attached cells		
	chemical	radiation + chemical	chemical + radiation
1	2	3	4
- (control)	42.5±2.5	-	-
- (irradiated sample)	64.5±3.1	-	-
1. Arachidonic acid, 1x10^{-5} M	25.5±2.1*	68.0±3.2§	27.4±6.2
2. SOD[1]), 25 U/ml	30.0±4.1*	73.1±3.1§	31.6±2.2
3. Ouabain, 7x10^{-4} M	16.8±3.3*	36.6±2.1§	17.8±1.4
4. Catalase, 2600 U/ml	27.2±5.1*	57.2±2.1§	40.2±4.3§
5. SOD, 250 U/ml	26.7±5.1*	55.9±3.2§	23.9±1.3
6. Ouabain, 7x10^{-5} M	27.1±2.2*	51.6±1.0§	27.1±2.1
7. Sodium azide, 1x10^{-4} M	23.6±2.1*	53.6±1.1§	38.7±3.3§
8. ATP, 5x10^{-5} M	21.0±2.1*	38.0±3.0§	21.0±1.4
9. Rotenone, 1x10^{-5} M	28.0±4.3*	43.5±3.3§	26.3±5.2
10. DNP, 2x10^{-5} M	28.0±7.0*	34.4±3.1§	30.5±2.1
11. Quinacrine, 6x10^{-4} M	17.2±5.0*	25.6±4.1§	63.8±2.2§
12. Melatonin, 4x10^{-5} M	26.5±5.2*	36.1±7.2§	26.1±5.1
13. Mannitol, 2x10^{-3} M	34.4±7.4*	51.6±3.0§	37.1±4.1
14. GSH, 1x10^{-5} M	29.2±5.0*	35.7±3.0§	23.6±4.2
15. H_2O_2, 1x10^{-3} M	16.3±6.2*	19.8±2.1§	29.1±3.1§
16. Amiloride, 5x10^{-4} M	69.6±5.9*	91.3±4.0§	69.2±3.1
17. Methylene blue, 1x10^{-3} M	59.6±6.0*	75.6±3.0§	64.0±5.215
18. GSSG, 1x10^{-4} M	40.0±4.1	70.1±3.0§	25.4±1.3
19. $NaNO_2$, 4x10^{-4} M	42.1±2.5	58.3±2.1§	43.1±2.2
20. SNP, 5x10^{-4} M	36.1±3.3	32.3±1.0	15.7±4.3§
21. 2-ME, 2x10^{-4} M	42.5±5.0	36.6±5.0	29.8±2.1§
22. Cysteine, 1x10^{-4} M	42.0±2.0	33.0±8.0	6.5±2.2§
23. $CuSO_4$, 2x10^{-5} M	40.7±2.1	39.0±3.1	41.8±6.6

*- significant from the control;
§- significant from the action of chemical
1) - Abbreviations: SOD-superoxide dismutase
DNP- dinitrophenol
ATP- adenosin triphosphate
GSH- glutathione
GSSG- glutathione disulphide
SNP- sodium nitoprussid
2-ME- 2-mercaptoethanol

to the analysis are only those pairs of samples whose pre-irradiation increases the attached cell percentage (items 1 through 19 in Table 3), the correlation coefficient proves higher, namely, 0.6207 ($P < 0.0001$) (Figure 8).

Comparison between the data listed in columns 2 and 4 of Table 3 indicates that the difference in attached cell percentage between the post-irradiated samples (column 4) and simply chemical-treated samples (column 2) is in most cases statistically not significant. However, there are four chemicals (catalase, sodium azide, quinacrine, and H_2O_2, respectively numbered 4, 7, 11, and 15 in Table 3) with which the attached cell percentage in the post-irradiated samples (column 4) is statistically significantly higher than that in the simply chemical-treated samples (column 2). The chemical quinacrine (item 11 in Table 3) is a special case. Post-irradiation in the case of this chemical increases cell attachment strongly (form 17.2±5% to 63.8 ±7.2%). This differs from the action of the other chemicals. Pre-irradiation reduces the cell-attachment inhibition caused by quinacrine by merely a few percent (from 17.2 ±5% to 25.6±4.1%).

There are also three chemicals (SNP, 2-ME, and cysteine, respectively numbered 20, 21, and 22 in Table 3) with which the attached cell percentages in the post-irradiated samples are lower in comparison with the controls.

The linear regression analysis of the data listed in columns 2 and 4 of Table 3 indicates that there is no correlation between them, the correlation coefficient being equal to 0.1147 ($P = 0.1140$).

So, it was found that irradiating HeLa cell suspension samples at $\lambda = 820$ nm prior to their being treated with certain chemicals reduces (or even completely eliminates in some cases) the cell-to-glass adhesion inhibition caused by these chemicals. The existence of a positive and statistically significant correlation between these two types of treatment (Figure 8) suggests that there might be a more general phenomenon at work. No correlation has been found to exist in the case where the cells are irradiated after their being treated with the chemicals. However, the chemicals tested are not too many, and what is more, they have been specially chosen for the way they act on the various metabolic pathways in the cell. Also, no studies have been conducted to reveal the concentration dependence of the action of these chemicals. On the other hand, recall that the pre-irradiation of HeLa cells with a monochromatic red light reduces their cytotoxic response to high doses of γ-radiation. It has been suggested in this case that pre-irradiation modulates the cell metabolism in such a way as makes the cells less susceptible to the subsequent radiation damage [44]. In some cases, genetical effects are suggested to be involved [45,46,57].

The primary events in cells exposed to visible-to-near IR radiation are believed to occur in their

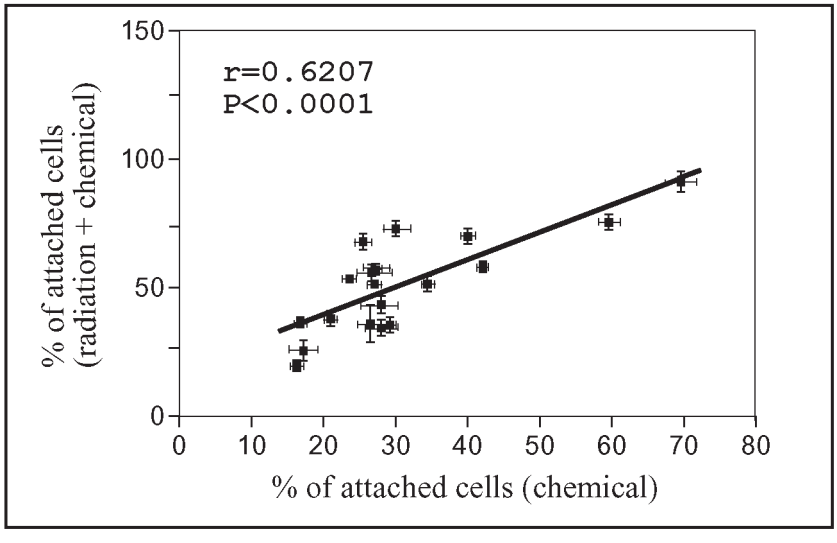

Fig. 8. Linear regression analysis of attached cell percentage data for the cells irradiated prior to their being treated with chemicals and the cells treated with chemicals. The analysis covers 19 pairs of samples (items 1 through 19 in Table 1, columns 2 and 3) with statistically significant difference between the respective values in columns 2 and 3 (marked by the symbol §).

mitochondria (cytochrome c oxidase is supposed to be the photoacceptor involved). The cellular membrane is part of the photosignal transduction and amplification chain (or cellular signaling cascade) between the mitochondria and the nucleus (review [5]). This suggestion is supported by the similarity between the action spectra for the DNA and RNA synthesis rates [5] and those for cell adhesion [52]. The action spectrum for cell adhesion is presented in Fig. 7 (top right-hand corner). Also, the increase of cell adhesion is dose-dependent (Fig. 7). Integrins, as well as focal adhesion molecules, which regulate and mediate between the cell-matrix interactions, belong in the large class of glucoproteins that do not absorb the near-IR radiation used in the present research. The effect of irradiation at 820 nm on cell adhesion is apparently not associated with the direct action of light on these molecules.

If the primary photoacceptor is located in the mitochondrion, while the process of interest involves the plasma membrane (adhesion), then how is the photosignal transmitted between the organelles? The answer is that this occurs by way of cellular signaling. The cellular signaling pathway (or photosignal transduction and amplification chain) in our case is supposed to start with the respiratory chain including the cytoplasm, the plasma membrane, and the nucleus. The way the signal is transmitted is supposed to be a cascade of rapid changes in cellular homeostasis parameters, crucial among them being the redox potential and ATP content of the cell [5].

Irradiation supposedly causes the cellular redox balance to shift toward a more oxidized state (slight oxidative stress), it also optimizes the energy status of a cell [5]. In our experiments, we have used six groups of chemicals that may affect cellular signaling in different ways. These include antioxidants, respiration inhibitors, NO donors, chemicals influencing the phospholipase A_2 (PLA_2) pathway, chemicals blocking the monovalent ion channels in the plasma membrane, and thiol-reactive chemicals. However, one should keep in mind the fact that the effect of chemicals on living cells is multiform. This circumstance complicates the interpretation of modulation experiments with chemicals.

The antioxidants (free radical scavengers) used in our experiments (melatonin, mannitol, sodium azide, SOD, catalase) inhibit cell adhesion (column 2 in Table 3). From among these chemicals, SOD and catalase are incapable of penetration because of their high molecular weight. With all of the antioxidants, pre-irradiation increases the cell attachment suppressed by them. The respiratory chain inhibitors rotenone (acting at the level of NADH-dehydrogenase) and sodium azide (acting at the level of cytochrome c oxidase), as well as the ionophore DNP (electron flow and ATP synthesis decoupler), also inhibit cell adhesion, and pre-irradiation improves the situation here (Table 3). Thus, one can suppose that the improvement of cell attachment (reduction of the adhesion suppression effect, normalization of cell adhesion to the control level, or its elevation above the latter) by pre-irradiation is due to the antagonistic actions of the radiation and the chemicals used on cell attachment. In other words, the slight oxidative stress caused by the radiation diminishes the subsequent effect of the chemicals.

Methylene blue that subverts the electron flow in the respiratory chain increasing the superoxide anion production, increases cell attachment (column 2, Table 3), and pre-irradiation augments this effect (column 3, Table 3). In that case, the radiation and the chemical act in the same direction, i.e., both of them cause some oxidative stress.

The action of the two inhibitors of the monovalent ion flows through the plasma membrane, amiloride (Na^+/H^+ antiporter inhibitor) and ouabain (Na^+, K^+- ATPase inhibitor), is similar to that of the chemicals in the preceding groups. The difference in action between these two chemicals is that amiloride itself stimulates cell attachment (column 2, Table 3). With amiloride and ouabain alike,

pre-irradiation causes the attached cell percentage to grow higher (column 3, Table 3).

The action of radiation in cell attachment modulation for the remaining three groups of chemicals is not so clear. Both the NO donors, $NaNO_2$ and SNP, have no effect on cell attachment (column 2, Table 3). Pre-irradiation has a strong positive effect in the case of $NaNO_2$ and not in the case of SNP (column 3, Table 3).

The effect of pre-irradiation is remarkably strong in the case of arachidonic acid (attached cell percentage rise from 25.5±2.1% to 68.0±3.2%, Table 3). The attached cell percentage in the pre-irradiated sample (68.0±3.1%) is comparable with that in the irradiated sample (64.5±3.1%). Arachidonic acid is a lipid messenger involved in the integrin-modulated cell attachment and spreading. It is released from the membrane phospholipids, predominantly by PLA_2 [58]. At the same time, the treatment of cells with the PLA_2 inhibitor quinacrine diminishes the number of the attached cells (column 2, Table 3). Irradiation of the cells following their treatment with this chemical stimulates their adhesion equally as does irradiation only (attached cell percentages 63.8±2.2% and 64.5±3.1%, respectively, Table 3). One explanation of this finding may be that the PLA_2 - arachidonic acid pathway is not involved in the radiation-induced cellular signaling process. But to prove or disprove this suggestion requires other experimental approaches.

The thiol-reactive chemicals cysteine, $CuSO_4$, and 2-mercaptoethanol have no effect on cell attachment under our experimental conditions. GSH that inhibits cell adhesion (item 14, column 2 in Table 3) is an exception. Pre-irradiation has practically no modulation effect on the percentage of the attached cells treated with cysteine, $CuSO_4$, and 2-ME (column 3 in Table 3). However, in some cases (cysteine, 2-mercaptoethanol) post-irradiation reduces cell attachment significantly (columns 2 and 4 in Table 3). Thiol-reactive chemicals can cause the cellular redox balance to shift toward a more reduced state. Also, they can directly react with integrins and other cell adhesion molecules. At the moment we can conclude that the action of this group of chemicals in our experimental conditions differs from that of the chemicals in the other groups (antioxidants, respiratory chain inhibitors, inhibitors of the monovalent ion flows).

Thus, the pre-irradiation of a suspension of HeLa cells at a wavelength of $\lambda = 820$ nm reduces or completely eliminates the cell attachment inhibition caused by the antioxidants, respiratory chain inhibitors, and inhibitors of the monovalent ion flows through the plasma membrane. For the thiol-reactive chemicals and NO donors, the effect of pre-irradiation is not pronounced. A correlation ($r = 0.6207$, $P < 0.0001$) has been found to exist between the percentage of the attached chemical-treated cells and that of their counterparts in case of irradiation preceding chemical treatment.

5. Concluding remarks

By light interaction with a biotissue, coherent properties of laser light are not manifested at the molecular level. The absorption of low-intensity laser light by biological systems is of a purely non-coherent (i.e., photobiological) nature. On the cellular level, the biological responses are determined by absorption of light with photoacceptor molecules. Coherent properties of laser light are not important when cellular monolayer, thin layer of cell suspension as well as thin layer of tissue surface are irradiated. In these cases, the coherent and noncoherent light with the same wavelength, intensity and dose provides the same biological response. Some additional (therapeutical) effects from the coherent and polarized radiation can occur only in deeper layers of bulk tissue (Section 2).

The data provided in Section 3 clearly shows that possible long term effects of irradiation (i.e., the changes in cellular metabolism occurring in cells, which ancestors were irradiated) should be

investigated before low power laser therapy will become a mainstream medical tool.

The data from Section 4 demonstrates a possibility of low-power laser radiation preventing and eliminating toxic effects of some chemicals (antioxidants, respiratory chain inhibitors, and inhibitors of the monovalent ion flows through the plasma membrane). This potentially beneficial use of low power (laser) light clearly needs further experimental investigations.

I would like to finish this chapter with some words about two recent papers [59,60]. These papers provide experimental evidence that regulation of gene expression includes pathways that detect energy levels (redox levels) and repress DNA transcription when cellular NADH levels are increased. Important point from these papers in connection with cellular mechanisms of low-power therapy is the following. First, two transcription factors (clock: BMAL1 and NPAS2:BMAL1) that control gene expression as a function of the light-dark cycle are regulated by the redox state of NAD cofactors [59]. In other words, the authors suggest that circadian clock may be entrained by direct modulation of cellular redox state. Many years ago it was suggested that effects of (laser) light on cellular metabolism may be connected with an action on existing cellular periodicities, i.e., with clocks (pp. 143-146 and 165-168 in [3]). The data presented in [59] supports this early suggestion [3] about possible link of low-power laser cellular effects with endogeneous rhythmicity oscillator via modulation of cellular redox state [3]. Also, recently neurohormone melatonin, which is primarly connected with clocks, was found to modulate near IR radiation action on HeLa cells [61].

Second point of interest in connection with cellular mechanisms of low power laser therapy is that the binding of the corepressor carboxyl-terminal binding protein (CtBP) that is involved in transcriptional pathways important for cell cycle regulation, was found to be redox-regulated by NAD^+ and NADH [60]. It means that the ability to detect changes in nuclear NAD^+/NADH ratio allows CtBP to serve as a redox sensor for transcription. It was found years ago that in case when cellular populations are irradiated, one of the effects of He-Ne laser radiation is the regulation of cell cycle (reviews [3,6]). So, the data from recent papers [59,60] supports the hypothesis from 1988 [4,2] that a crucial step in cellular effects of low power laser irradiation is a transient modulation of cellular redox potential caused by activation of respiratory chain.

The data from [59,60] evidences that there are many still unknown ways how metabolic pathways in a cell are regulated. No wonder that mechanisms of light regulation of cell metabolism (cellular mechanisms of low-power laser therapy) are understood only fragmentary yet.

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Curriculum Vitae

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Born and educated in Tartu (Estonian Republic). Received the diploma in physical chemistry at Tartu University in 1970. Graduated in photochemistry and chemical carcinogenesis with Academician L.M.Shabad at National Cancer Research Centre of USSR (Moscow). Ph.D. in 1974, Doctor of Sciences (biophysics) in 1990. Last 20 years the main scientific interests have been in the field of laser light-tissue interaction. The author of 2 books ("Photobiology of Low Power Laser Therapy", 1989; "The Science of Low Power Laser Therapy", 1998) and almost 300 papers. More about CV and scientific activities, as well as selected publication list can be found in

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Avocations: classical music, opera, gardening, brush writing, golf. Married to Prof. V.S.Letokhov. Daughter Inga is MD and works as the anesthesiologist in Tallinn (Estonia).