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Effects of Monochromatic Low-Intensity Light and Laser Irradiation on Adhesion of HeLa Cells In Vitro

Tiina 1. Karu, PhD, Ludmila V. Pyatibrat, MS, Galina S. Kalendo, PhD, and Rinat 0. Esenaliev, PhD

Laser Technology Center (T.I.K., L. V.P.) and Institute of Spectroscopy (R.O.E.), Russian Academy of Science, 142092 Troitsk, Moscow Region, Russia; Cancer Research Center, Russian Academy of Medical Science, 114578 Moscow, Russia (G.S.K.)

Background and Objective: **The adhesion of HeLa cells was evaluated after irradiation with monochromatic low-intensity light or laser irradiation. It is well known that the cell—cell and cell—matrix adhesion changes during wound repair. For better understanding of low-power laser light action on the wound healing process, it would be of interest to study the light action on cellular adhesion in vitro.**
Study Design/Materials and Methods: **The monochomatic light was in the range 580-860 nm (bandwidth 10 nm, 5-150 J/m^2 1.3 W/m^2) and the He-Ne laser irradiation was 632.8 nm (100 J/m^2,10 W/m^2). Cell-cell and cell-glass adhesion were evaluated after irradiation of HeLa cells.**
Results: **It was found that cell-cell and cell-glass adhesion increased following irradiation depending on the irradiation conditions (wavelength, dose) and the time elapsed after the irradiation. The cell attachment to glass surface increased after irradiation of samples of HeLa cells in suspension.** ***Conclusion:*** **The adhesion was stimulated in the wavelength ranges 600-625,645-700, and 720-850 nm with maxima at 620,680, 750, and 820-830 nm, respectively.** © **1996 Wiley-Liss, Inc.**

Key words: cell-cell adhesion, cell-glass adhesion, low-power laser therapy, wound-healing

INTRODUCTION

Low-power visible and near IR (laser) irradiation is claimed to stimulate wound healing and tissue regeneration. Changes in proliferation and motility of fibroblasts [1-4] and keratinocytes [5,6] in vitro, as well as changes in tensile strength of irradiated wounds in vitro [7-9] after irradiation, have been described. Both cellular migration and proliferation have particular dependence on adhesion. During wound repair, the cells also use various adhesive strategies (both in cell-cell and cell-matrix relationships) to respond appropriately to a changing environment [10,11].

There have been few investigations into adhesive properties of cells in vitro after laser irradiation. He-Ne laser radiation was found to increase attachment of fibroblasts to the growth substrate [I]. Plating efficiency of HeLa cells was increased in a dose-dependent way after He-Ne laser irradiation [12]. He-Ne laser irradiation also enhanced adherence of salmonella to lymphocytes [13]. In particular, the irradiation increased the number of lymphocyte receptor sites.

The aim of the present experiments was to investigate changes both in cell—cell and cell-growth substrate adhesion after irradiation with monochromatic visible and near IR light. Dependencies of adhesion strength on the time elapsed after irradiation, as well as on the dose and wavelength of the light, were measured. The experi-

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Address reprint requests to Dr. T.I. Karu, Laser Technology Center of Acad. Sci., 142092 Troitsk, Moscow Region, Russia.

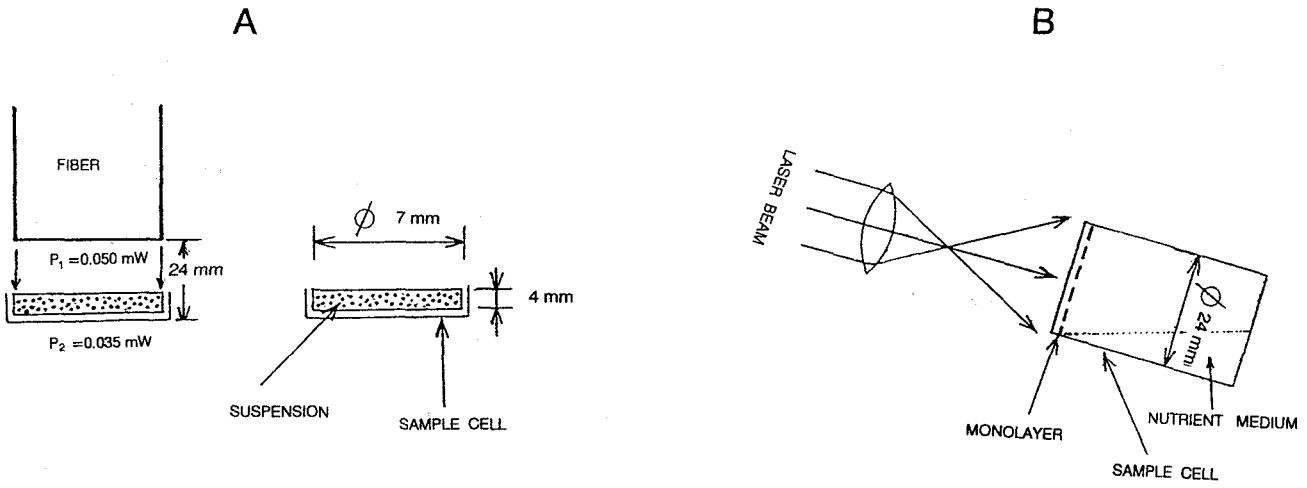

Fig. 1. Irradiation of (**A**) cellular suspension, (**B**) cellular monolayer.

ments were performed with HeLa cells. Earlier, an increase in proliferation after irradiation with monochromatic visible and near IR light was demonstrated on the same model [12, 14—16].

MATERIALS AND METHODS

Cells

HeLa cells, initially obtained from the Institute of Virulogy, Moscow, Russia, were grown in scintillation vials in 2 ml medium 199 with the addition of 10% bovine serum and 100 units/ml of penicillin and streptomycin. The experiments were performed 72 h after plating exponential phase of the growth [12]. Cell viability was assessed by trypan blue exclusion test, and all experiments were performed with cell suspensions demonstrating >85% viability by this criterion. Details about the cultivation and growth of the culture can be found elsewhere [12, 16].

Light Sources and Irradiation

Monochromatic radiation was obtained by means of a monochromator designed by Dr. Lifshits at the Institute of Spectroscopy, Russian Academy of Sciences, to have bandwidths of 400-700 nm and 540-1,050 nm. The monochromator was operated in accordance with an autocolloma-tion scheme. A single-slit 1.2 mm wide was used to direct radiation both into and out of the mono-chromator. Dispersion amounted to 8 nm/mm. Thus the exit radiation bandwidths came to 10 nm (FWHM, full wide at half maximum). The dispersive element used was a diffraction grating of 1,200 lines per millimeter. To obtain radiation in the near IR region, the second order of the grating, a thermal filter (H_2O) and a glass filter, placed between the radiation source (250 W high-pressure, xenon-arc lamp) and the monochromator, were used. The source radiation was focused with a positive lens and delivered by a fiber light guide to the upper part of the slit. After diffraction of the beam, the monochromatic radiation was exited from the lower part of the slit and transmitted by another light guide. The well with the sample of the cellular suspension (S = 0.38 cm^2) was covered completely (Fig. 1A). In the range of the wavelengths 580-860 nm, the output power of the monochromator and the power measured after the sample (P_1 and P_2 in Fig. 1A) were constant (0.050 mW and 0.035 mW, respectively). Measurements were made by Spectra Physics M404 power meter. Light intensity used in experiments was 1.3 W/m^2.

Cells were grown in monolayer during 72 h, then carefully harvested using 0.02% Versene solution, and suspension was prepared in medium 199 containing 10% of bovine serum. 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 grinded edges (inner diameter 0.7 cm/and height 0.4 cm) were stuck on a microscope slide with 5 cm distance between them. Both wells were filled with cell suspension (85,000 viable cells per vial, volume 130 (μl). One of the vials was irradiated and another was used as control. The cell suspension exactly filled the vial to avoid formation of the meniscus. Optimal irradiation conditions (shape and dimensions of the vial, number of cells

per vial) were developed in a special series of experiments. Irradiation was performed at room temperature in the dark.

For irradiation of the cellular monolayer (72 h after the plating, 2.5-10^5 cells/vial), He-Ne laser (λ = 632.8 nm, Spectra Physics model 125A) was used. The beam was expanded with a short-focus positive lens to a diameter of 24 mm, which corresponded to the diameter of the bottom of the vial covered with the monolayer (Fig. 1B). Neutral filters were used to reduce the intensity of light to 10 W/m^2. The irradiation time was 10 s, and the fluence, 100 J/m^2. These parameters were found earlier to be optimal for proliferation activation of HeLa cells grown in monolayer [14—16]. Spectra Physics M404 power meter was used for measurements of light intensity. The vial was placed into a special holder and remained closed during the irradiation. The monolayer was not covered with the medium due to the position of the vial (Fig. 1B). Irradiation was performed in the dark through the bottom of the vial. The controls were treated exactly by the same way (except the irradiation procedure).

Measurement of Cell-Cell Adhesion

Cellular monolayers were irradiated as described earlier, and at definite times after the irradiation the strength of cell-cell adhesion was estimated by counting the number of single cells and cell complexes after a standard procedure of dispersion of the monolayer. The standard dispersion procedure included the following stages: onefold rinse of the monolayer with warm (37°C) 0.02% Versene solution, incubating the mono-layer in 1 ml of Versene solution during 5 min at 37°C, and then carefully pipeting with 30 strokes using standardized pipets. The cellular suspension was analysed by counting single cells and cellular complexes (2 or 3 cells in complex, complexes containing >3 cells were not found). The data are presented as mean ±SEM of at least five experiments with three vials per point per experiment.

Measurement of Cell-Glass Adhesion

Immediately after irradiation, the glass slide with samples of cell suspension (Fig. 1A) was put into the thermostat (37°C) for 30 min (if not stated in other ways). Then, the nutrient medium was removed and the glass wells were washed with Hanks' solution to remove all nonattached cells. The attached cells were trypsinized and their number was counted by a hemocytometer. The results presented are the average mean ±SEM at least of three experiments with five vials per point per experiment.

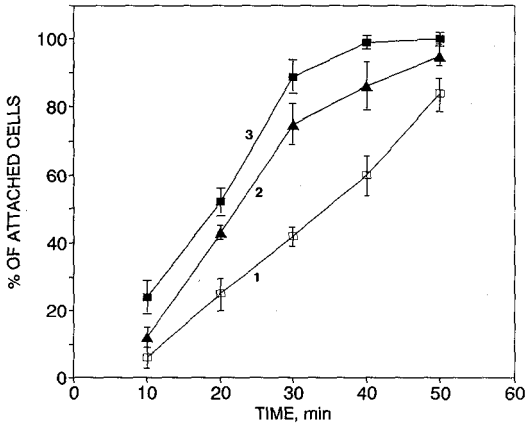

Fig. 2. Rate of cell attachment to glass: (1) nonirradiated suspension, cellular suspension irradiated (2) at 620 nm, 52 J/m^2 or (3) at 680 nm, 52 J/m^2.

RESULTS

In the first series of experiments, we investigated cell-glass (growth substrate) adhesion after the irradiation of freshly prepared cell suspension. The suspension was irradiated during 40s (52 J/m^2) with the light of various wavelengths, and the number of attached cells was measured at certain time intervals elapsed after the irradiation. The results are expressed as a percentage of viable cells originally present in the sample cell. Figure 2 shows some examples of kinetics of cellular attachment to the glass. As seen in Figure 2, the percentage of attached cells depends both on time interval elapsed and the wavelength used for the irradiation. The percentage of attached cells also depends on the dose of the light as illustrated in Figure 3. Figure 3 presents data about cellular attachment for various wavelengths and doses when the time elapsed after the irradiation was constant (30 min).

As the next step, dependence of the percentage of attached cells on wavelength (action spectrum) was recorded in the following conditions:
the dose 52 J/m^2 (1.3 W/m^2, 40 s) and the time elapsed after the irradiation, 30 min. As seen in Figure 4, the cell attachment to the glass surface was observed to be stimulated upon irradiating the cellular suspension sample with light in the wavelengths ranges 600-625, 645-700, and 720-

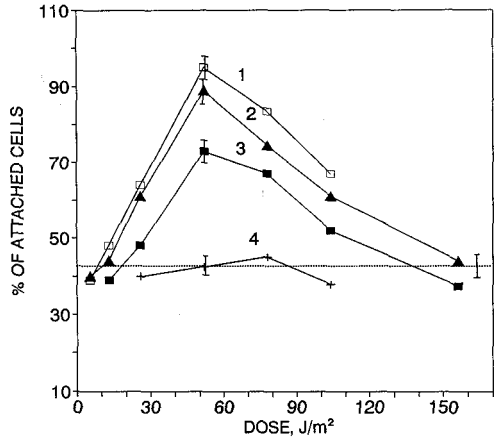

Fig. 3. Cell attachment to glass as dependence on the light dose after irradiation of cellular suspension with the following wavelengths: (1) 830 nm, (2) 680 nm, (3) 620 nm, (4) 700 nm. Measurements were made 30 min after the irradiation. Dashed line marks attachment of nonirradiated suspension.

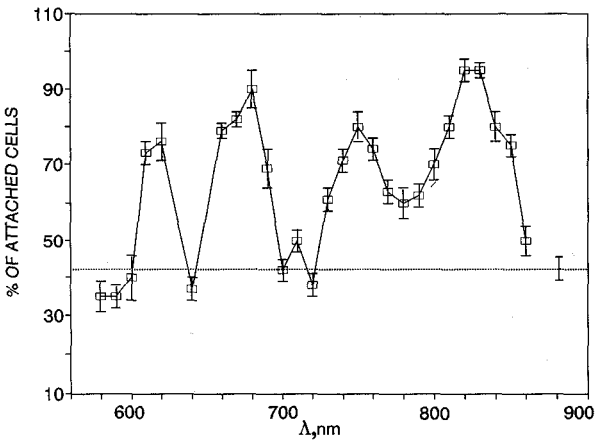

Fig. 4. Cell attachment to glass as dependence on wavelength used for irradiation of the samples suspension (action spectrum). D = 52 J/m², I = 1.3 W/m², t = 40 s, measurements were made 30 min after irradiation. Dashed line marks attachment of the nonirradiated suspension.

850 nm with maxima at 620, 680, 750, and 820-830 nm, respectively.

In the experiments described above, effect of the irradiation on the ability of cells to attach to the growth substrate was measured. In other words, the action of irradiation upon new contacts between the cell membrane and glass was studied. The next question of interest is whether the irradiation also can influence the strength of the cell-cell attachment, i.e., the contacts that were formed before the irradiation. To answer this question, in the second series of experiments the cells were irradiated in the monolayer. Changes in the cell-cell adhesion strength were evaluated as described earlier.

In the control (unexposed cells), the composition of the suspension prepared by the standard procedure of the dispersion of monolayer was always kept at the same level: (89.6 ± 0.9)% of individual cells, (7.6 ± 0.5)% of cell complexes consisting of two cells, and (4.6 ± 0.6)% of cell complexes consisting of three cells. The complexes consisting of more than three cells were not found in control or in irradiated samples. In the suspensions prepared from irradiated monolayers, the percentage of individual cells as well as cellular complexes depended on the time elapsed after the irradiation (Figs. 5,6). Recall that the irradiation lasted 10 s and after that, the monolayer was in the nutrient medium at 37°C. The monolayers were dispersed at certain time points shown in the abscissa in Figures 5 and 6. Figure 5 presents the data about changes in percentage of individ ual cells, and separate cellular complexes from two and three cells. Figure 6 summarizes data about changes in percentage of cellular complexes. The data in Figures 5 and 6 are evidence that after irradiation the strength of cell-cell adhesion varies with the time elapsed. During the first minutes after irradiation, the cell—cell adhesion increases and reaches a maximum after 30 min and then drops to the control level. A new increase in cell-cell adhesion begins at longer time intervals after the irradiation.

DISCUSSION

The cell surface and its adhesive interactions are involved in regulation of such processes as embryogenesis, cell growth and differentiation, morphogenesis, and formation of metastasis [17-23]. In spite of the fundamental character of these processes, their importance in biology and medicine is limited, inasmuch as the understanding of molecular mechanism of adhesion is still fragmentary. Inadequate knowledge of cellular adhesion mechanism is partly conditioned by lack of adequate methods to measure the adhesive strength.

The specific affinity is the result of the summation of different adhesion systems. Some of the adhering molecules have been isolated and characterized [20-22]. Both cell-cell adhesion and cell-matrix adhesion molecules isolated and characterized so far belong to glycoproteins, proteoglycans, and glycolipids [22], i.e., to the classes of

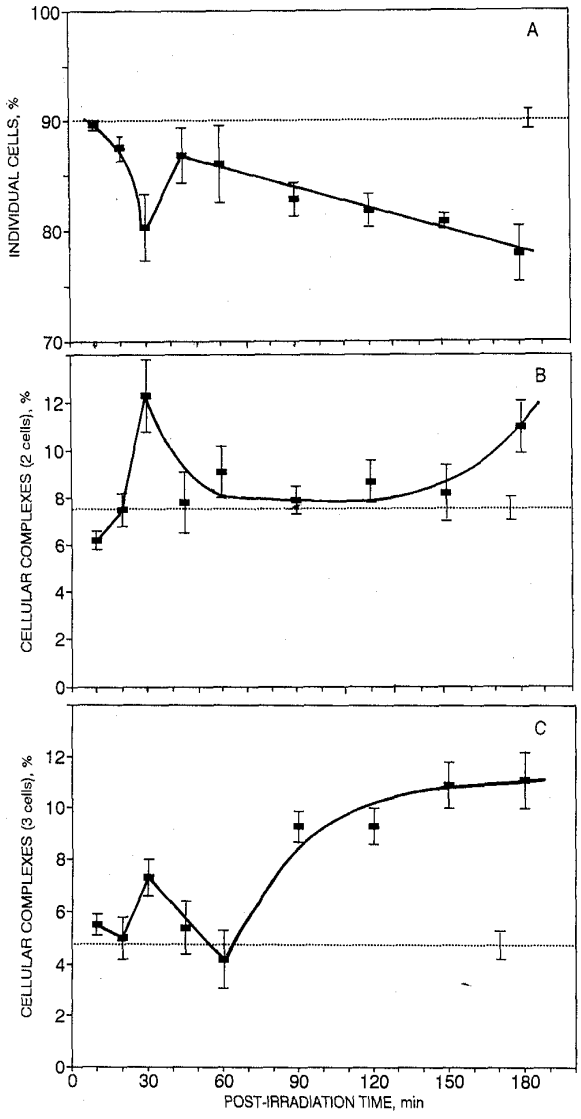

Fig. 5. Changes in number of (**A**) individual cells, (**B**) cellular complexes of two cells, and (**C**) cellular complexes of three cells at various times elapsed after the irradiation of monolayer with He–Ne laser (632.8 nm, 100 J/m^2, 10 W/m^2). Dashed lines denote the respective levels in case of nonirradiated cells.

molecules that do not absorb visible and near IE light [24]. In other words, they probably change adhesive properties of cell membrane described in the present work not due to primary excitation of some of the adhesion molecules.

Biological responses of cells to monochromatic (laser) light occur due to electronic excitation of photoacceptor molecules (which are suggested to be components of the respiratory chain) [25]. These physical and chemical reactions are fast and occur under the light action (primary re

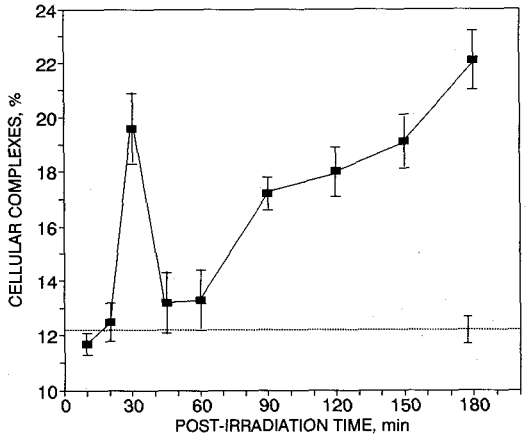

Fig. 6. Dependence of cell–cell attachment strength on time elapsed after irradiation of the monolayer with He–Ne laser (632.8 nm, 100 J/m^2, 10 s). Dashed line denotes the percentage of cellular complexes after dispersion of nonirradiated monolayer.

actions). The primary physical and/or chemical changes in photoacceptor molecules are followed by a cascade of biochemical reactions in the cell that do not need direct light activation and occur in the dark in the time scale of minutes and hours after the irradiation (secondary reactions forming a photosignal transduction and amplification chain) [26]. Figure 7 presents a possible mechanism of light-induced changes in proliferation. As seen in right part of Figure 7, the signal from electronically excited photoacceptors located in the mitochondria is amplified and transduced to the nucleus by a cascade of biochemical reactions in cytoplasm and cell membrane (which is important from the point of view of the present work). The left part of Figure 7 shows the photosignal transduction and amplification chain. The end points of the photosignal transduction and amplification chain (photobiological responses) are changes in the DNA and RNA synthesis rate.

The action spectra of monochromatic visible and near IR light on DNA and RNA synthesis were recorded [14, 15]. Figure 8 depicts these two action spectra together with the action spectrum recorded in the present work for the cell attachment. As seen in Figure 8, the three action spectra are rather similar in respect to the location of the maxima: 610-620 nm, 680 nm, 750-770 nm, and 820-830 nm. Considering the width of the light bands used for the irradiation (10 nm FWHM in case of curve 1 and 14 nm FWHM in the case of curves 2 and 3, [14,15], one can conclude that the maxima in the three action spectra have

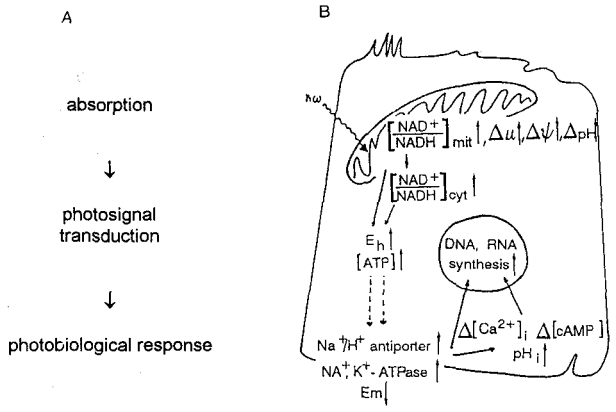

Fig. 7. A possible scheme of proliferation changes of in mammalian cells by monochromatic visible (laser) light: (**A**) photosignal transduction and amplification chain together with (**B**) respective biochemical reactions in a cell. Light is absorbed in mitochondria and stimulation of DNA and RNA synthesis (photobiological response) occurs as a final step after a cascade of biochemical reactions (photosignal transduction and amplification) in a cell. The details of the scheme are described in [25].

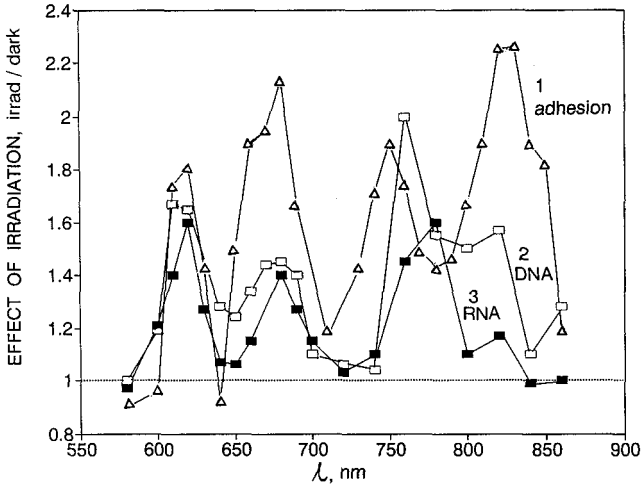

Fig. 8. Action spectra for: (1) cell–glass adhesion, (2) DNA synthesis, and (3) RNA synthesis in HeLa cells after the irradiation of cells with visible and near IR monochromatic light. The absolute values can be found in Figure 4 (for curve 1) and in [14, 15] (for curves 2, 3). Errors bars are ≤ 0,1 arbitrary units. Bandwidth of the monochromatic light (FWHM) is 10 nm (curve 1) and 14 nm (curves 2, 3).

practically the same location. This finding certainly supports the suggestion that these three reactions have common primary photoacceptor(s) (Fig. 7) [5]. It is known that an action spectrum provides, within certain limits, a representation of the absorption spectrum of the photoacceptor pigment [27].

One should note that the finding of existence of the action spectrum for such a complex and multiphase process like cell-glass adhesion was surprising and unexpected. Cells from freshly prepared cell suspension will sediment toward the glass under the influence of gravity. During early phases of the adhesion (occurring approximately in the first 30 min), the round-shape cells only attach to the growth substrate but do not spread [17, 20]. Recall that in our experiments we measured the number of the attached but not spread cells. It is known that early phases of cell adhesion are accompanied by intensification of ion fluxes through the membrane [20]. Ionic gradients are known to have an important role in early stages of transmembrane signalling and proliferation control [28]. Therefore, it might be expected that irradiation also modulates ion gradients and reactions involved in cell adhesion. This suggestion is supported by experimental data evidencing that reagents affecting ion transport across the cell membrane also enhance or diminish cell sed-imention and adhesion [29, 30]. It is also known that systems regulating intracellular pH (e.g., Na^+/H^+ antiport through the cell membrane) are activated by cell adhesion to solid substrate [31]. Recall that a jump in intracellular pH is one of the steps in the photosignal transduction chain (Fig. 7). To explain the dependence of cell adhesion on the wavelength used for the irradiation (action spectrum), the following hypothesis can be advanced. One can suggest that intensity of ion fluxes through the cell membrane depends on intensity of the signal received from the photoacceptor, which in its turn depends on the primary reactions occurring in/with photoacceptor(s) during the irradiation with light at different wavelengths.

Summing up, the main observation in the present studies is that the irradiation with monochromatic visible and near IR light leads to enhancement of cell adhesion (both cell-cell and cell-glass relationships). The enhancement of cell adhesion depends on the irradiation conditions (dose, wavelength) as well as on the time elapsed after irradiation. Increase in cellular adhesion as a result of (laser) irradiation could probably be associated with positive findings in low-power laser therapy described in the literature [3, 7-9].

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