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Critical Review

Gene Expression Under Laser and Light-emitting Diodes Radiation for Modulation of Cell Adhesion: Possible Applications for Biotechnology

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Summary

Experimental data about the modulation of adhesion and proliferation of anchorage-dependent HeLa cells with monochromatic or quasimonochromatic radiation in red to near-infrared region are presented. Cell adhesion and proliferation can be increased by irradiation with light of certain wavelengths (maxima in action spectrum are 619, 675, 740, 760, and 820 nm) or decreased when the activity of photoacceptor (cytochrome *c* oxidase in mitochondrial respiratory chain) is inhibited by chemicals before the irradiation. This modality allows controlling the number of attached and/or proliferating cells. Possible biotechnology applications of this method are outlined. © 2011 IUBMB

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Keywords modulation of anchorage-dependent cell adhesion and proliferation; monochromatic and quasimonochromatic red to near-IR radiation; retrograde mitochondrial signaling; charge-transfer and d–d transitions in Cu chromophores of cytochrome *c* oxidase molecule.

INTRODUCTION

Adhesion is the ability of cells to attach to a surface (first step in anchorage-dependent growth) or to each other in the case of anchorage-independent growth. This property of cells and its modulation are important for various biotechnology applications. Anchorage-independent cell growth is preferred in many bioengineering applications to get a large amount of cells quickly. However, in some applications, anchorage-dependent cell growth is preferable. For example, adhered cells can be used to create addressable platforms of microarrays of attached cells, which can be used for drug screening applications (*1–4*). It would be beneficial to manipulate with cell adhesion to extracellular matrices insofar as this process is the initial event in cell proliferation *in vitro*. Proliferation is also an important cellular property for biotechnology, referring to the rate at which cells grow after the adhesion and divide after passing through the different cell cycle phases (*2, 5*).

Anchorage-dependent growth of cultured cells (both the attachment of cells to the matrix and their passing through cell cycle) can be increased by laser and light-emitting diodes irradiation in red to near-infrared (near-IR) optical region, the effects depending on the wavelength used for the irradiation as well as on the radiation dose (*6*). The aim of this review is to summarize the experimental data about light-dependent modulation of cell adhesion and proliferation as well as to outline possible biotechnology applications.

There are genomic differences between cell lines with anchorage-independent and anchorage-dependent growth. For example, there are two distinct cell lines of HeLa cells (tumor cells of human origin, a widely used model system in cell biology), characterized by anchorage-dependent or anchorage-independent growth with an existing broad list of differently expressed genes. Anchorage-dependent HeLa cells exhibit a lower expression of the *siat7e* gene, and a higher expression of the *lama4* gene relative to anchorage-independent HeLa cells. Either by decreasing the expression of the *siat7e* or by enhancing the expression of *lama4* in anchorage-dependent HeLa cells, an increase in cellular adhesion can be observed (*5*). The *lama4* gene product is a complex glycoprotein that mediates adhesion, migration, and development by interacting with other extracellular matrix components. The *siat7e* gene product is involved in glucosylaton. Additional studies have highlighted a number of genes thought to be involved in mediating adhesion such as

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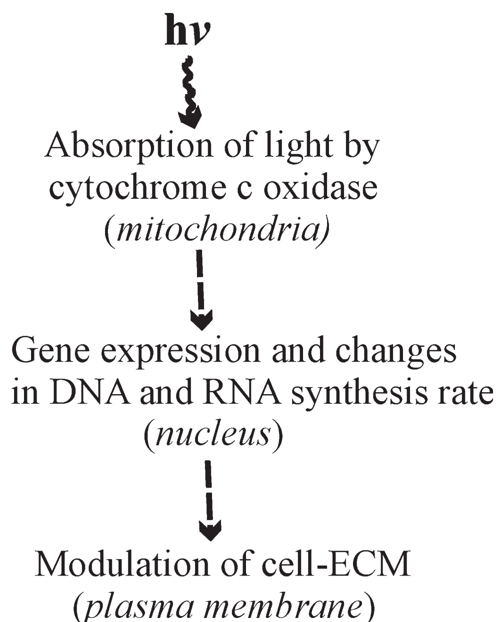

Figure 1. A schematic explaining the principle of mitochondrial retrograde signaling after absorption of monochromatic and quasimonochromatic visible and near-IR radiation (marked *hv*) by the photoacceptor, cytochrome *c* oxidase.

rhoA, *rac1*, and *cdc42* (*5*). The example about HeLa cells is given here, because our experimental work referred below was performed on anchorage-dependent HeLa cells.

MODULATION OF CELL ATTACHMENT BY MONOCHROMATIC RADIATION IN RED TO NEAR-IR OPTICAL REGION AND VARIOUS CHEMICALS: RETROGRADE MITOCHONDRIAL SIGNALING

Cell adhesion to an extracellular matrix or artificial substrata is mediated by members of the integrin family (*7, 8*). Integrin receptors initiate numerous signal transduction pathways that control cell shape, motility, proliferation, and death (*8, 9*). Most of the different integrins are expressed in a variety of cell types, and most cells express several integrin heterodimers, allowing them to adhere to many different extracellular matrix (ECM) molecules (*9*).

The integrins and focal adhesion molecules belong to the large family of glycoproteins (*9*) that do not absorb light of red to near-IR spectral region. It means that in the case when cell adhesion can be modulated by irradiation with light in this spectral region, there should exist another photoacceptor molecule.

It is believed that modulation of cell attachment induced by the irradiation of cells with monochromatic and quasimonochromatic radiation in the visible-to-near IR region involves a retrograde-type photosignal transduction chain that starts from the photoacceptor located in the mitochondria, cytochrome *c* oxidase (*10*). This information channel between the mitochondrial respiratory chain and the nucleus transduces signals regarding the functional state of the mitochondria. Radiation of visible and near IR (IR-A) radiation can activate a retrograde-type cellular signaling pathway between mitochondria and the cell genome (*11*). A simple schematic explaining changes in cellular metabolism after absorption of visible and IR-A radiation (marked *hv*) by the photoacceptor, cytochrome *c* oxidase, is presented in Fig. 1. A more detailed scheme can be found in the literature (*10, 12*). It should be emphasized that this signaling pathway, as well as its possible modulation, has not been well studied until recently. It is thought that a modulation of retrograde mitochondrial signaling may allow the modulation of the life or death of cells, and even aging (*13*).

Figure 2A presents the dependence of per cent of adhered anchorage-dependent HeLa cells on wavelength used for the irradiation (the action spectrum). The bands in the action spectrum of attachment stimulation of anchorage-dependent HeLa cells, as well as in action spectra of enhancement of DNA and RNA synthesis rate of these cells, were identified by analogy with the metal–ligand system absorption spectra characteristic of the visible-to-near IR spectral range (*15*). This analysis allowed the conclusion that all bands in these three similar action spectra may be related to cytochrome *c* oxidase. These spectra are characterized by one maximum at 400 nm with the edge of the envelope near 450 nm (not shown in Fig. 2A), by a single peak at 619 nm, peak at 675 with a weak shoulder at 699 nm, doublet peaks at 740 and 760 nm, as well as peak in near IR region at 820 nm, with shoulders at 800 and 840 nm. Bands at 675 and 820 nm were attributed to a relatively oxidized form of cytochrome *c* oxidase. The edge of the blue-violet band at 450 nm and the distinct bands at 619 and 760 nm are supposed to belong to a relatively reduced form of the enzyme (*15, 16*). The main contribution to the 619 nm band in the spectrum in Fig. 2A is believed to arise from the reduced Cu_A, to the 675 nm band from the oxidized Cu_B, to the 760 nm band, from the reduced Cu_B, and to the 820 nm band, from the oxidized Cu_A chromophores of cytochrome *c* oxidase. The bands at 619 and 675 nm are mainly due to charge transfer from ligands to copper atoms; the bands at 760 and 820 nm are due to d–d transitions inside the copper atoms (*15*). The d–d transition means that the extra energy provided by a photon causes electronic excitation between two d sublevels of the Cu atom.

It was suggested that the photoacceptor is a yet unidentified intermediate of the redox cycle of cytochrome *c* oxidase that appears in turnover (*15, 17*). The exact peak positions for every action spectrum can be found in Table 1. Later, the action spectra of cell adhesion and DNA and RNA synthesis rate were compared with the absorption spectrum of a cell monolayer in the region of 600–860 nm. A comparison between the absorption and action spectra provided evidence that all bands present in the action spectra were also present in the absorption spectra of cellular monolayers (*17*). The importance of the action spectra for a practical use in biotechnology is not only making conclusions about mechanisms of adhesion but also these spectra indicate the optimal wavelengths providing possible maximal

effects. The action spectrum in Fig. 2A shows that the optimal ranges of wavelengths to increase the number of attached cells were 615–630 nm, 660–680 nm, 730–770 nm, and 810–880 nm. The optimal dose range in the same experimental conditions was 40–110 J/m^2. In case of the irradiation of cell suspensions, one has to take into account the thickness of the cell layer, as well as concentration of cells, to avoid a strong attenuation of light. It means that the optimal dose range can differ from that shown above. The optimal wavelength ranges do not depend on these circumstances.

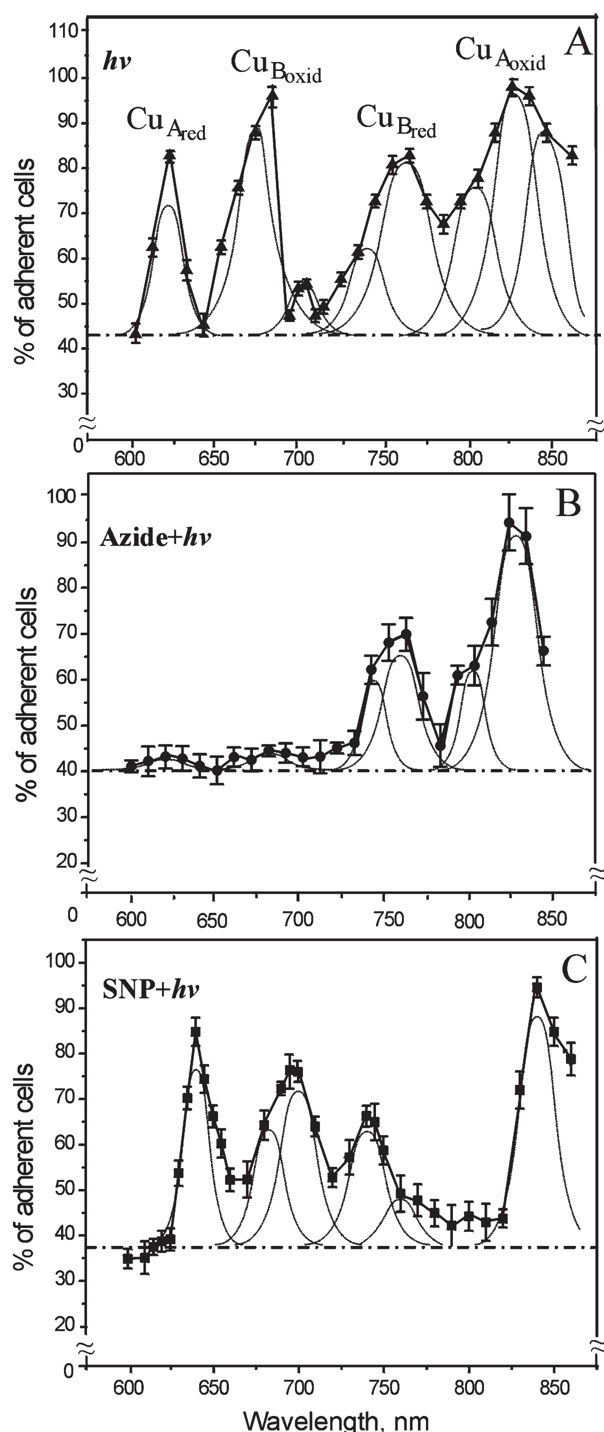

Figure 2.

The earlier experimental investigations of the modulation of cell adhesive properties by low-power (laser) light provided evidence that the plating efficiency (*20, 21*) and cell motility (*22*), as well as the adherence of *Salmonella* to lymphocytes (*23*) increased after irradiation with monochoromatic red light of various wavelengths. The cell–glass attachment was found to be dependent on the dose (*6*) and wavelength of the continuous wave (CW) 600–860 nm light (*16*) (see also Fig. 2A), and on the pulse parameters when the cells were irradiated with 820 nm pulse-modulated light (*24*). The irradiation at 810 nm did not reveal a significant increase in adhesion of periodontal ligament cells *in vitro* (*25*). Low doses of ruby (694 nm), alexandrite (755 nm), and Nd:YAG laser irradiation at 1,064 nm (*26*) as well as at 532 nm (*27*) induced changes in cell adhesion by modulating the integrin expression pattern (*26*) and the focal adhesion kinase activity (*27*).

A large-scale experiment was performed to inhibit various cellular signaling pathways with chemicals of known action mechanisms. The dependencies of number of adhered cells on wavelength and dose as well as the modulation of these dependencies with various chemicals were studied to explore cellular signaling pathways between mitochondria, plasma membrane, and the nucleus (*28–32, 14, 18, 19*). The chemicals tested included inhibitors of the respiratory chain, antioxidants (free-radical scavengers), NO donors, and inhibitors of phospholipase A_2, inhibitors of the flow of monovalent ions through the plasma membrane, oxidants, and thiol-reactive compounds (*28–32, 14, 18, 19*). Manipulating phenotypic behaviors of cells is a powerful tool for modifying the properties of cells for the desired biotechnology objective (*4*).

Experiments in which the modulation of mitochondrial electron transport chain was performed using chemicals with known action mechanism indicated that chemicals, which act at different points of the respiratory chain, inhibited cell attachment and

Figure 2. (A) The dependence of number of attached cells on wavelength (light action spectrum) when HeLa cell suspensions were irradiated (CW light, D = 52 J/m^2, I = 1.3 W/m^2, τ = 40 s, adhesion measurements performed 30 min after the irradiation). The action spectra when the following chemicals were added before the irradiation to cell suspension: (B) sodium azide (2×10^{-5} M) or (C) SNP (5×10^{-5} M). The dashed lines in A–C indicate the attachment of cells: (A) as without irradiation or (B,C), in the presence of the respective chemical without irradiation. The fine lines indicate Lorentzian fitting (adapted from ref. *14*, with permission from John Wiley & Sons). $Cu_{A_{red}}$, $Cu_{B_{oxid}}$, $Cu_{B_{red}}$, and $Cu_{A_{oxid}}$ mark the chromophores of cytochrome *c* oxidase responsible for absorption of visible-to-near IR radiation in respective spectral bands.

Table 1
The peak positions in control action spectrum of anchorage-dependent HeLa cells as well as the peak positions in light action spectra when sodium azide or SNP was added before irradiation of cell suspension

Control spectrum		Azide added (2×10^{-5} M)		SNP added (5×10^{-5} M)	
Chromophore of cytochrome *c* oxidase molecule putatively responsible for absorption in main band	Peak positions (nm)	Peak positions (nm)	Changes in comparison with control spectrum	Peak positions (nm)	Changes in comparison with control spectrum
$Cu_{A_{red}}$	**619**	–	Disappeared	–	Disappeared
				642	New peak with the same intensity
$Cu_{B_{oxid}}$	**675**	–	Disappeared	685	Shift + 10 nm and decreased intensity
	699 w. sh., not resolved	–	Disappeared	**700**	New peak
$Cu_{B_{red}}$	740	745	No changes in peak position and intensity	**742**	New distinct band with the same intensity
	760	**760**	No changes in peak position and intensity	~760 w.sh., not resolved	From this peak only a shoulder keeps
$Cu_{A_{oxid}}$ $Cu_{A_{oxid}}$	800 w. sh.	800 w. sh.	No changes in position and intensity	–	Disappeared
	820	**825**	No changes in peak position and intensity	–	Disappeared
	840 s.sh.	–	Disappeared	**842**	No changes in peak position and intensity

Peak positions were calculated from Lorentz fit data; changes ≤5 nm were considered to be nonsignificant. Main peaks are marked bold (Data adapted from refs. *18*, *19*, and *14*, with permissions from RSC Publishing, John Wiley & Sons, and John Wiley & Sons, respectively); w. sh.: weak shoulder, s. sh.: strong shoulder.

prevented the attachment stimulation caused by the irradiation (*18, 19*). The chemicals tested were dinitrophenol (an uncoupling agent that abolish the obligatory linkage between the electron transport and the phosphorylation system), rotenone (blocks NADH dehydrogenase in complex I of the respiratory chain), sodium azide (blocks cytochrome *c* oxidase catalytic center permanently), and nitric oxide (blocks cytochrome *c* oxidase catalytic center reversibly dependently on oxygen concentration, and works also as a potential signaling molecule controlling cell respiration).

The action of two chemicals, sodium azide and sodium nitroprusside (SNP, a NO donor), which regulate the activity of cytochrome *c* oxidase (the photoacceptor molecule), was examined comparatively. Azide bridges the heme of cytochrome a_3 and Cu_B permanently, but NO binds to the catalytic center of cytochrome *c* oxidase reversibly. The complicated dependencies of cell attachment on irradiation dose at different concentrations of NO donors, as well as dependencies of cell attachment on the concentration of various NO donors when irradiating at a constant dose, which were studied in separate experiments (*14*) indicated that both factors (irradiation and NO) had a subtle influence on a reaction channel (cellular signaling cascade) between the photoacceptor (cytochrome *c* oxidase) and cell response to the irradiation (attachment in our case; ref. *14*). Figures 2B and 2C show the action spectra, when sodium azide or SNP (a NO donor) was added before the irradiation.

The concentration of sodium azide used in this experiment (2×10^{-5} M) did not influence cell attachment without irradiation (40.8 ± 2.5%, marked by dotted line in Fig. 2B, vs. 42.5 ± 2.5% in control, Fig. 2A). A comparison of this action spectrum (Fig. 2B) with the control spectrum (Fig. 2A) shows that the most dramatic changes occur in the red region (peak at 619 nm is fully eliminated), as well as in the far red region. The main peak at 675 with a shoulder at 699 nm in the control spectrum is also fully eliminated (Table 1 and Fig. 2B). This finding means that charge transfer complexes to $Cu_{A_{red}}$ and $Cu_{B_{oxid}}$ are closed for electron transport in the presence of sodium azide. There are practically no changes (as compared with control spectrum) in electron transport connected with the suggested d–d transitions in the $Cu_{B_{red}}$ chromophore (characterized by doublet bands at 745 and 760 nm), and only few changes in electron transport connected with the suggested d–d transitions in the $Cu_{A_{oxid}}$ chromophore in the near-IR region occur (disappearance of the shoulder at 840 nm, Table 1).

Figure 2C shows the measured action spectrum when SNP (5×10^{-5} M) was added to cell suspension samples before the irradiation. SNP at a concentration of 5×10^{-5} M causes a statistically nonsignificant inhibition of cell attachment without irradiation (37.8 ± 2.1% vs. 42.5 ± 2.5% in control). This new action spectrum is characterized by a single peak at 642 nm, doublet peaks at 685 and 700 nm (main peak), single peak at 742 nm (with a weak shoulder at 760 nm), and with a peak at 842 nm. Two charge transfer channels putatively to $Cu_{A_{red}}$ and $Cu_{B_{oxid}}$, as well as two reaction channels putatively connected with d–d transitions in $Cu_{B_{red}}$ and $Cu_{A_{oxid}}$, were reorganized in the presence of NO (Fig. 2C and Table 1). An analysis of the data presented in Fig. 2C and Table 1 indicates that NO causes a new internal distribution of electrons in redox centers of cytochrome *c* oxidase. An important feature of this rearrangement is closing some reaction channels between redox centers and opening some new ones with practically similar characteristics (*e.g.*, band intensity).

For possible biotechnology applications when there is a need to regulate cell attachment, irradiation with monochromatic light in the red to near-IR region or the irradiation of cells with a NO donor or azide present can be used for cell attachment stimulation or its inhibition, respectively. Recent results using cDNA microarrays indicate that irradiation with red light regulates genes related to mitochondrial energy metabolism (*33*).

INCREASE OF CELL PROLIFERATION BY THE IRRADIATION

Biotechnology is one of the areas, which stimulated extensive research into the control of the mammalian cell cycle. One area of interest in cell cycle studies has always been the regulation of the G_1–S and G_2–M transitions, especially the arrest in G_1 cell cycle phase (including the so called G_0 phase or quiescence). Indeed, a decrease of the duration of G_1 (G_0) phase is the main possibility to shorten the cell cycle and in this way to quickly increase the amount of cells produced (*34*).

One gene encoding a cytochrome *c* oxidase subunit (*cox15*) was found to be regulated differently in anchorage-independent and anchorage-dependent HeLa cells. The *cox15* gene is upregulated in anchorage-independent HeLa cells relatively to anchorage-dependent HeLa cells (*2*). Cyclin-dependent kinase like 3 (*cdkl3*) gene, which takes part in cell transition from the G_0/G_1 phases to the S phase, was found to be upregulated in both anchorage-dependent and anchorage-independent HeLa cells (*2*). A short-time He–Ne laser (λ = 632.8 nm) irradiation increased the expression of cell-cycle regulatory proteins cyclin D_1, cyclin E, and cyclin A in mouse satellite cells (*35*). The activation of ERK1 (extracellular signal regulated kinase 1), which is needed for the continued expression of cyclin D_1 in the G_1 phase of the cell cycle, occurred after induced by irradiation with a He–Ne laser cell proliferation (*36*). The irradiation with a He–Ne laser also upregulated the expression of proliferating cell nuclear antigen in the late G_1 phase of primary rat satellite cells (*37*). These findings certainly suggest that the irradiation influences expression of early cell-cycle regulatory genes and causes proliferation increase. It was also found that irradiation with red light induces a cell cycle progression from G_1 to S phase through the redistribution and degradation of promyelytic leukemia protein (*38*). This topic has been reviewed recently (*33*).

The experimental data referred to above show that a modulation of the activity of the photoacceptor (cytochrome *c* oxidase) by irradiation with monochromatic and quasimonochromatic

radiation in the red to near-IR spectral region, and regulating cellular signaling pathways in this way, can modulate cell proliferation rates.

Autoradiographic experiments with irradiated (He–Ne laser; λ = 632.8 nm) anchorage-dependent HeLa cell monolayers show that cell proliferation was changed. The number of S-phase cells was increased (enhanced G_1–S transition of a part of cell population) as well as the grain count on the labeled nuclei (enhanced DNA synthesis in S-phase cells ref. *21*). Different kinetics of these processes in irradiated cells and a comparison of the results of continuous-labeling and pulse-labeling experiments allowed the conclusion that the proliferative activity of slowly growing cell subpopulations was increased in the irradiated samples (*21*).

USING THE MICROARRAY TECHNIQUE FOR STUDIES ON LIGHT ACTION UPON CELL ATTACHMENT AND PROLIFERATION

For over a decade, DNA microarray technology has been used to gauge gene expression levels of an entire genome in a single experiment. This technology is nowadays used in evolutionary biology, disease characterization, diagnostics, and forensics (*4*). However, the first observation about changes in gene expression by irradiation of resting cells with red light was made before the advent of this technology. It appears that *c-myc*, a proto-oncogene that encodes a transcription factor, which is involved in the enhancement of cell proliferation (*39*), was upregulated in human lymphocytes when irradiated with 670-nm light (*40*). Both the irradiation at 670 nm and treatment of cells with mitogen phytohemagglutinin caused an increased accumulation of prematurely terminated proto-oncogene *c-myc* RNA, but not a variation in amount of full-length *c-myc* RNA.

The cDNA microarray analysis of expression profiles in human fibroblasts HS27 irradiated with red light at a wavelength of 632.8 nm was studied (*41*). This analysis revealed that 111 genes grouped into 10 functional categories were upregulated by irradiation. The 111 genes affected by red light belonged to the following categories according to their functions: cell proliferation, antioxidant relations, apoptosis or stress–response, metabolism-related channels transport, cytoskeleton and cell–cell interactions, DNA synthesis and repair, and transcription functions. Among these 10 functional gene categories, seven of them were directly or indirectly involved in cell proliferation. Two signaling pathways, the p38 mitogen-activated protein kinase signaling pathway and the platelet-derived growth factor signaling pathway, were found to be involved in cell growth induced by the irradiation of cells with red light at 632.8 nm. Several genes related to antioxidation and mitochondria energy metabolism were also found to express differentially upon irradiation. All the genes in the subcategory related to energy metabolism of the respiratory chain, were upregulated. These results allowed authors to conclude that increase in ATP synthesis after the irradiation may be caused by the upregulation of the expression of certain enzymes of the respiratory chain (*41*).

CONCLUDING REMARKS

Controlling the adhesion of anchorage-dependent cells is a multifaceted issue that takes into account the effects connected with the cell metabolism, as well as those connected with ECM molecules turnover. The effects caused by irradiation, for example, the enhancement of the number of attached cells, and an increase in proliferation rate are connected with the cells and their metabolism. Those effects can be controlled with appropriate irradiation parameters, and by modulating the attachment with chemicals via the photoacceptor, cytochrome *c* oxidase.

The irradiation of cells with light in the red to near-IR region cannot cause any harmful effects on cell metabolism even at high doses (*6*). However, using chemicals to modulate cell adhesion in combination with irradiation may in some cases be cytotoxic to cells, and also cause harmful effects to ECM in higher concentrations. Inasmuch as cells naturally produce and secrete endogenous matrix molecules, and remodel the ECM by secreting processes, the immobilized ECM can be degraded. However, these possible side effects are controllable.

.One possible useful development for biotechnology would be an upregulation of the expression of certain genes by irradiation for producing needed gene products. Also, defining the location of cells by controlling adhesion would be useful for positioning cells on the sensing elements of cell-based biosensors, as well as in microarrays of adherent cells that are used for drug screening applications.

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