

## EFFECTS OF LOW-POWER LASER IRRADIATION ON CELL PROLIFERATION

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### Abstract

In this study, the effect of low-power laser irradiation on the proliferation activity of HeLa cells was investigated. The cells were irradiated by a 830 nm semiconductor BTL-10 laser in a continuous or pulsed mode at an energy density ranging from 2 to 99 J/cm<sup>2</sup> (power output, 72 to 360 mW). The irradiated cells were incubated and their proliferation activity was assessed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay at 24, 48, 72 and 96 h. In comparison with the control populations, the irradiated cells showed a significant increase in proliferation, regardless of the energy density used, at 72 and 96 h but not at 24 and 48 h. In addition, the stimulation of proliferation was related to the mode of irradiation. The cells irradiated in the pulsed mode (5 000 Hz) showed a higher proliferation activity than the cells treated by continuous laser light. It is concluded that low-power lasers stimulate HeLa cell proliferation.

### Key words

Low-power laser, Cell proliferation, HeLa cells, MTT assay

### INTRODUCTION

Laser energy is generated on the principle of light amplification of stimulated emission of radiation. The beam of laser energy is highly coherent, polarised, focused and monochromatic (1). Its selectively destructive quality was first used in ophthalmology in the early 1960s, although the principle on which lasers are based was postulated by Einstein in 1917. Non-invasive or soft lasers were introduced into medicine in the 1980s and, since then, have gained wide application in many areas of health care (2). Non-invasive, low-power lasers with an output up to 500 mW have been reported to have stimulatory, anti-inflammatory and analgesic effects (3).

The transmission of laser radiation in tissues is related to its wavelength. Infrared laser radiation shows a higher penetration into tissues than the laser light of the red region of the visible spectrum. Therefore, the latter has proved useful in treatment of skin and mucosal disorders (3).

The stimulatory effects of low-power laser irradiation at the cellular and molecular levels have been shown by many studies. Laser light affects the mitochondrial respiratory chain by changing the electric potential of cell

membranes and, consequently, their selective permeability for sodium, potassium and calcium ions, or by increasing the activity of certain enzymes such as cytochrome oxidase and adenosine triphosphatase (4). It also increases DNA synthesis (5), collagen and pro-collagen production (6, 7), and may increase the cell proliferation (8, 9) or alter locomotory characteristics of cells (10). In contrast to these stimulatory effects, some investigators have found damaging or even destructive action of soft laser radiation. *Ocana-Quero et al.*, for instance, described a degenerative effect of He-Ne laser irradiation on bovine oocytes (11).

Although studies in both laboratory conditions and clinical settings have been numerous, the biochemical reactions induced by low-level laser irradiation are still poorly understood. This emphasises the need of more cellular research into laser biology (12).

The aim of this study was to assess the effect of low-energy laser light at the cellular level. Since cell proliferation is one of the basic manifestations of any living organism, an insight into factors affecting cell proliferation in response to laser irradiation may be important in terms of therapeutic applications of lasers. HeLa cells were selected because our previous study had dealt with the effect of infrared laser radiation on the assembly of microtubules in this experimental cell system (13).

## MATERIALS AND METHODS

### CELL CULTURE

The cells of a human epitheloid HeLa cell line were used. They were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 2 mM glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (PAA Laboratories, Austria) in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> at 37 °C. They were subcultivated three-times per week.

Before laser treatment, the cells were removed by trypsinisation and DMEM was added to achieve a concentration of  $8 \times 10^4$  cells/ml. From this starting suspension, 1 ml amounts were taken for irradiation. Each treated suspension was immediately diluted with DMEM to a final concentration of  $1 \times 10^4$  cells/ml and 0.2 ml aliquots were transferred to a row of eight wells in each of the four 96-well microtitre plates. Altogether ten suspensions exposed to different conditions of irradiation and a control non-irradiated cell population were assessed in each experiment carried out in triplicate. The suspensions in microtitre plates were incubated under standard conditions.

### IRRADIATION PROCEDURE

A BTL-10 semiconductor laser (Beautyline, Ltd., Prague, CR) emitting an 830 nm convergent beam was used in either a continuous or a pulsed mode (5000 Hz). The energy density ranged from 2 to 99 J/cm<sup>2</sup> and power output from 72 to 360 mW. The irradiation was carried out in four-well multidishes (Nunc, NUNC, Naperville, USA), containing 1 ml of the starting suspension in each well, with the probe situated perpendicular to the plate 0.5 cm above the cell suspension for irradiation times ranging from 27 to 275 sec (*Table 1*).

### PROLIFERATION ASSAY

The proliferation activity of cells was assessed using the MTT assay. This method is based on the ability of mitochondrial dehydrogenases in living cells to reduce soluble tetrazolium salts to

Table 1

Conditions of laser treatment of cell suspensions

Suspension no.	Energy density (J/cm <sup>2</sup> )	Power output (mW)	Frequency (Hz)	Time of irradiation (sec.)
1	99	360	0	275
2	99	360	5000	275
3	20	150	0	166
4	20	150	5000	166
5	12	72	0	133
6	12	72	5000	133
7	4	120	0	33
8	4	120	5000	33
9	2	72	0	27
10	2	72	5000	27
Control	—	—	—	—

a blue formazan product whose amount is directly proportional to the number of living cells. One of four plates was measured at 24, 48, 72 and 96 h after irradiation, respectively, by the following procedure: A 0.2 ml of MTT (final concentration, 0.456 mg/ml) was added to each well. The plate was incubated for 4 h at 37 °C. Subsequently, the medium with MTT was replaced with 0.2 ml dimethyl sulphoxide (Sigma, USA) in order to solubilise the formazan produced. The plate was directly scanned and measured in a Spectra Shell microplate reader (SLT, Austria) at 570 nm.

#### STATISTICAL METHODS

The results of cell number measurements were statistically analysed and presented as box-whisker plots with medians, and 25% and 5% quantiles for the distribution of values. The Mann-Whitney U test was used for comparing irradiated and non-irradiated suspensions and the data were expressed as the mean standard deviation. The values obtained from three independent experiments were homogeneous and therefore were summed up for the final analysis. The data were evaluated at the level of significance  $\alpha = 5\%$ . Both the calculations and graphs were processed by Statistica 6.0 software (StatSoft, Inc., USA).

#### RESULTS

The growth curves of all cell populations were similar regardless of the conditions of laser irradiation and showed a course presented in *Fig. 1*. At 24 h and 48 h there were no differences in cell proliferation between the irradiated and non-irradiated populations, whereas at 72 and 96 h these differences were significant.

An increase in absorbance at 72 h was significantly higher ( $P < 0.05$ ) in laser-treated suspensions than in the control cells; there was also a difference between the cells treated by the same energy density but in different modes of irradiation.

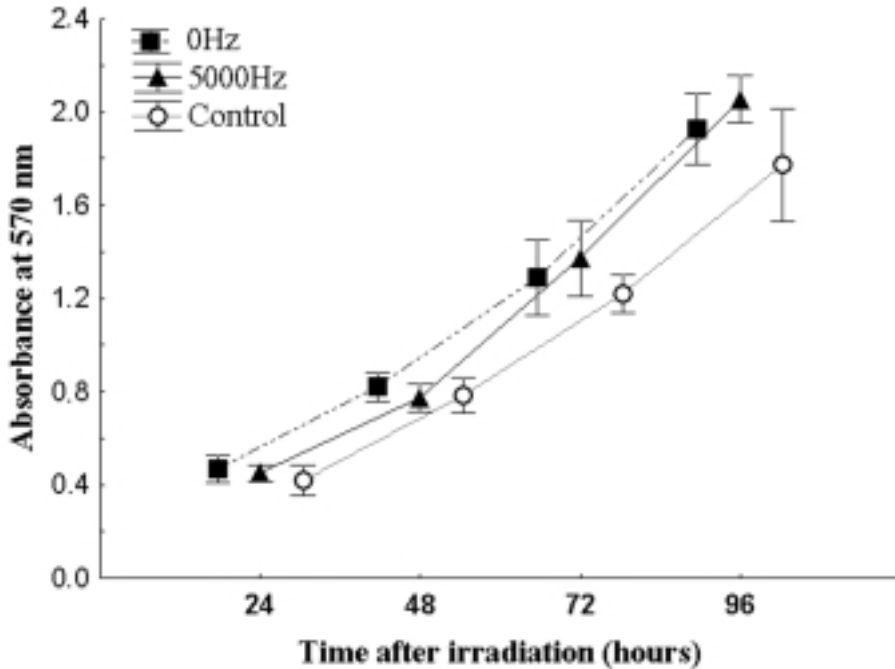


Fig. 1

Proliferation activity of HeLa cell suspensions after irradiation by laser light at a density of  $2 \text{ J/cm}^2$  in pulsed and continuous modes. Evaluated by the Mann-Whitney U test with values presented as meansSDs.

Irradiation in the pulsed mode (5000 Hz) stimulated cell proliferation more than irradiation in the continuous mode at all energy densities used, with the exception of  $12 \text{ J/cm}^2$ . At this density, cell proliferation increased more when subjected to the continuous mode (Fig. 2).

At 96 h the proliferation activity was significantly higher in the treated populations than in the non-treated cells ( $P < 0.05$ ). In all cultures, the pulsed laser light was more effective in stimulating proliferation than the continuous one applied at the same irradiation dose (Fig. 3)

#### DISCUSSION

The stimulatory effects of lasers on cell proliferation have been studied by many authors. Skin fibroblasts treated by laser energy densities of 2 and  $12 \text{ J/cm}^2$  at 670 nm achieved the maximal viability at 2 h and then at 3 days after irradiation

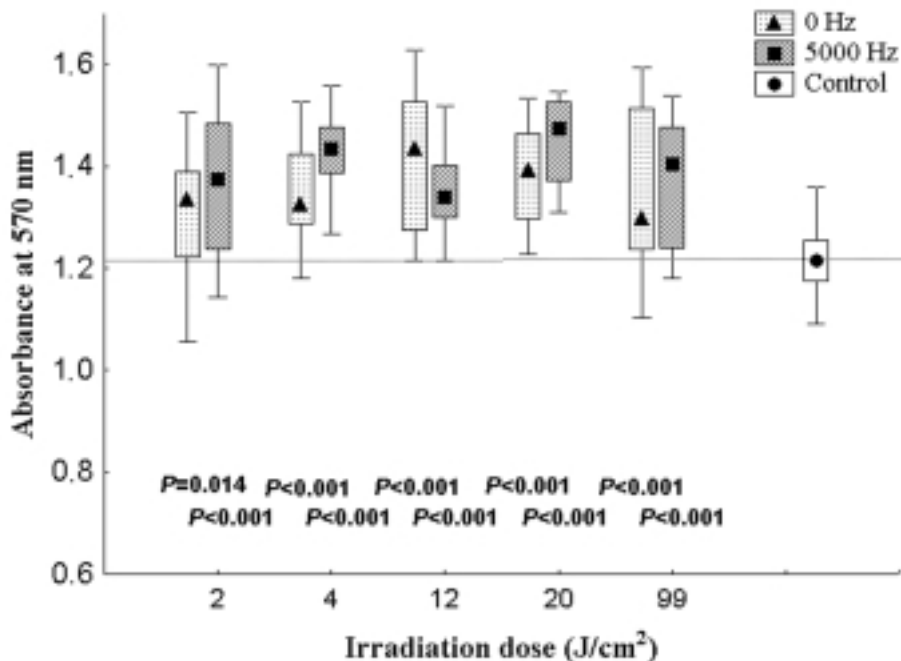


Fig. 2

Proliferation activity of HeLa cell suspensions at 72 h after laser irradiation at different energy densities. The values are presented as box-whisker plots with medians; box, 25 % and 75 %; whisker, 5 % and 95 %.

(8). In gingival fibroblast culture irradiated at 809 nm (energy densities from 1.96 to 7.84 J/cm<sup>2</sup>) the proliferation activity increased by 24 h but decreased thereafter (12). He-Ne lasers at a power output of 3.03 mW have stimulated the proliferation activity of osteoblast cultures, with a significant increase at 2 and 3 days after irradiation (14). In contrast, Haas *et al.* (15) did not find any proliferative effect of He-Ne laser irradiation and Abergel *et al.* (16) reported that, in fibroblast cultures, both He-Ne and Ga-Al-As lasers stimulated the production of pro-collagen but had no effect on proliferation.

In the C2 (murine myogenetic muscle) cells, the stimulation of proliferation has been related to the irradiation dose. At a wavelength of 805 nm, an energy density of 4 J/cm<sup>2</sup> has stimulated cell proliferation whereas 20 J/cm<sup>2</sup> has had an inhibitory effect (17). In the same study, MCF7 (human breast carcinoma) and U373 (human glioblastoma) cell lines were treated by laser light at 630 nm,

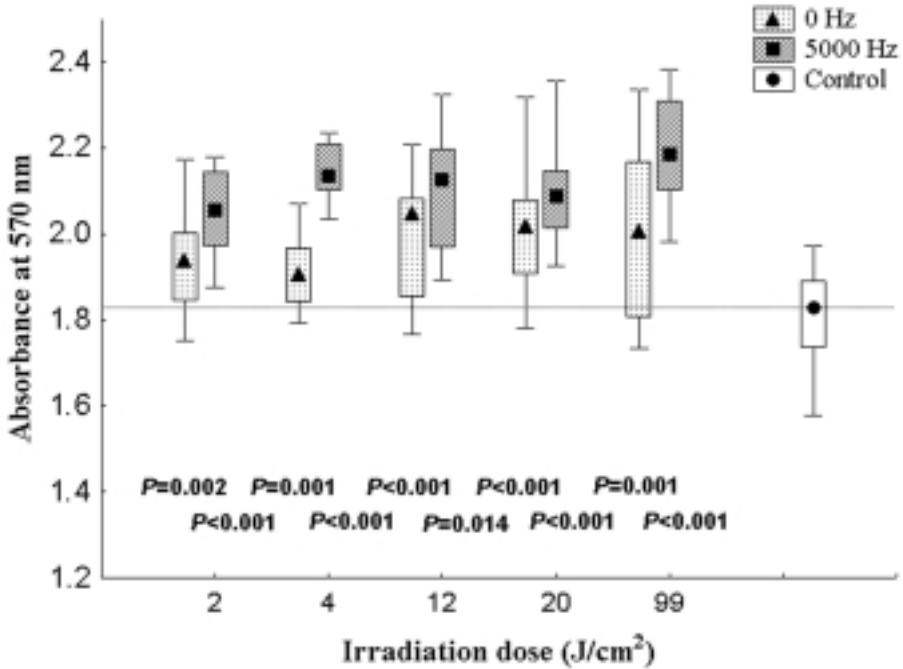


Fig. 3

Proliferation activity of HeLa cell suspensions at 96 h after laser irradiation at different energy densities. The values are presented as box-whisker plots with medians; box, 25 % and 75 %; whisker, 5 % and 95 %.

635 nm and 805 nm, using energy density in the range of 2 to 8 J/cm<sup>2</sup>. Cell proliferation was stimulated only by low doses. The stimulatory effect of low energy doses has also been studied in fibroblast cultures. The energy density of 3 and 4 J/cm<sup>2</sup> did increase cell growth but that of 5 J/cm<sup>2</sup> failed to promote proliferation. The source of irradiation in that study was a Ga-As laser at 904 nm (18). In our experiments, the proliferation of HeLa cells was stimulated by laser light of the near-infrared region (830 nm) at a much higher density, i.e., in the range of 2 to 99 J/cm<sup>2</sup>. This implies that both the type of cell and the type of laser are important factors in cell proliferation studies.

Our results concerning the effect of an irradiation mode on cell proliferation are in agreement with those of Šidlová (19). She irradiated A431 cell cultures (human epidermal cells) at a constant energy density of 12 J/cm<sup>2</sup> (830 nm), using different frequencies. The pulsed mode at 5000 Hz resulted in a higher

proliferation of cells assessed at 96 h after irradiation than the continuous mode. *Ueda and Shimizu (20)* demonstrated that even a low increase in frequency, i.e., from 0 to 1 Hz, affected the stimulatory action of a Ga-Al-As laser (830 nm, 50 mW) on the proliferation of osteoblast cultures. The fact that similar results have been achieved with infrared lasers in different cell cultures suggests that the mode of laser light application may play a more important role in the outcome of laser treatment than the wavelength of the laser used.

The effect of low energy laser light on cell proliferation has been accounted for by the involvement of photoreceptors in cellular processes leading to enhancement of cell growth (21). *Karu (22)* proposed a theory, later corroborated by *Friedmann and Lubart (23)*, that laser light is absorbed by components of the respiratory chain, such as flavin dehydrogenase, cytochromes and cytochrome oxidase, which results in respiratory chain activation and NAD-pool oxidation and, subsequently, in changes in the redox status of both mitochondria and cytoplasm. This may affect membrane permeability with a subsequent change in the  $\text{Na}^+/\text{H}^+$  ratio and an increase in  $\text{Na}^+$  and  $\text{K}^+/\text{ATP}$ -ase activity, which influences the  $\text{Ca}^{2+}$  flux. This is involved in the production of cyclic nucleotides that modulate DNA and RNA synthesis and, eventually, cell proliferation.

It has been suggested that cytochrome oxidase is a target for the absorption of light from the 800 to 830 nm region of the spectrum and, therefore, the respiratory chain is the starting point of any photoinduction effects elicited by infrared laser light (24, 25).

It can be concluded that the laser light of the near-infrared region (830 nm) stimulates cell proliferation but to a varying degree according to the irradiation dose and cell culture used. In HeLa cells, its effect is exerted over the range of energy densities from 2 to 99  $\text{J}/\text{cm}^2$ . The stimulatory effect is also influenced by the irradiation mode, with the pulsed light being more effective.

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#### VLIV LASERU S NÍZKÝM VÝKONEM NA PROLIFERACI BUNĚK

#### S o u h r n

V této studii byl zkoumán vliv nízkovýkoného laserového záření na proliferační aktivitu buněčné linie HeLa (nádorové buňky karcinomu děložního čípku). Zdrojem záření byl polovodičový laser BTL-10 s konstantní vlnovou délkou 830 nm aplikovaný v kontinuálním nebo pulsním režimu. Byla použita energetická hustota záření v rozsahu 2–99  $\text{J}/\text{cm}^2$  (výkon 72–360 mW, frekvence 0 a 5000 Hz). Proliferační aktivita byla stanovena za 24, 48, 72 a 96 hodin po počátečním ozáření buněk metodou MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Ve srovnání s kontrolními populacemi došlo u ozářených buněk po 72 a 96 hodinách

kultivace k signifikantnímu nárůstu bez ohledu na energetickou hustotu ozáření. Za 24 a 48 hodin nárůst buněk statisticky signifikantní nebyl. Navíc se ukázalo, že při stimulaci proliferace hraje úlohu použitý režim ozáření. Buňky ošetřené pulsním zářením (frekvence 5000 Hz) vykazovaly vyšší nárůst, než buňky ozářené v kontinuálním modu. Z výsledků vyplývá, že nízkovýkonné infračervené laserové záření je schopno zvýšit proliferaci buněk linie HeLa.

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