

LIGHT EFFECT ON FIBROBLAST PROLIFERATION

R. Lubart*, H. Friedmann[†], I. Peled[‡] and N. Grossman[‡]

* Department of Physics, Bar-Ilan University, Ramat Gan 52900, Israel.

[†] Department of Chemistry, Bar-Ilan University, Ramat Gan 52900, Israel.

[‡] Health Sciences Department, Ben-Gurion University, Beer-sheva 14105, Israel.

The healing effect of low energy lasers is generally attributed to enhanced cell proliferation due to the irradiation. As it was not clear whether coherent irradiation is essential, we examined the effect of various wavelengths from non-coherent light sources, on fibroblast proliferation. We found that light at 540 and 600–900 nm significantly accelerates the mitosis of these cells. Moreover, we have found that the effect is not only energy-dose dependent, but depends non-linearly on the intensity of the light source.

KEY WORDS Fibroblasts Proliferation Non laser light sources Non-linearity

Introduction

There has been much interest in the biological effects of low energy laser irradiation. Most of the experiments show that low energy lasers do have specific bioeffects which seem to change from stimulatory to damaging with increasing doses. In a recent paper¹ we irradiated fibroblasts with various lasers and found that at specific, relatively low energy dose there was acceleration of mitosis, while at higher doses the cells were destroyed. We then suggested^{1,2} that the effect of low energy laser irradiation in the visible and in the near infrared region was due to light absorption by either endogenous porphyrins in the mitochondria or by cytochromes. As the effect does not seem to be unique to lasers or to coherent irradiation, we irradiated fibroblasts with non-laser, non-monochromatic light sources and found that accelerated cell mitosis does occur at certain wavelengths and at a specific energy dose of non-coherent light. Moreover, we have found that the effect was not only dose dependent but also non-linearly intensity-dependent. These results have very important implications in phototherapy.

Materials and Methods

Materials

Culture media, fetal calf serum, trypsin and antibiotics were purchased from Biological Industries Co. (Beit Haemek, Israel). Tissue culture dishes were from Costar (Cambridge, Mass).

Fibroblasts

Dermal fibroblasts were established from explants from a four-year-old foreskin. The cells were grown in DMEM containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and gentamycin (50 µg/ml), at 37 °C and 8% CO₂.

Experimental Conditions

For each experiment, cells were seeded in 35 mm dishes, at $1.5 - 2 \times 10^4$ /dish. After 48 h, areas with comparable cell populations were marked on the culture dishes. The cultures were then irradiated, replenished with fresh growth medium, and were incubated for another 24 h.

The cultures were then washed with PBS, fixed with phosphate-buffered-formaldehyde, and the fraction of cells at mitosis was determined using an inverted microscope (CK2-TRC by Olympus, Japan).

Triplicate dishes were irradiated for each irradiation condition and three fields were counted in each irradiated area. Non-irradiated fields were counted as an internal control, and the mitotic values were similar to those obtained for non-irradiated dishes.

Irradiation

The light source was a tunable Xenon lamp with appropriate filters. The beam intensity was adjusted by the power supply of the lamp. An aperture was inserted into the optics to limit the illuminated field to 1.0 cm diameter. During the irradiation the cells were kept in their medium.

Results and Discussion

Human fibroblasts, in culture, were irradiated with non-laser light sources and the resultant alterations of the fraction of dividing cells were determined 24 h later. The first series of experiments showed that for each light source enhanced cell division was dose-dependent. The maximal effect of non-coherent light irradiation at 540 nm was obtained at 4 J/cm² (Figure 1). Irradiation with a broad band at 600–900 nm yielded maximal increase of the fraction of dividing cells, at 24 J/cm² (Figure 1). These results, which are in good agreement with previously obtained results¹ where the light sources were lasers, indicate that the monochromaticity or coherency of the light is not essential.

We have chosen the 540 nm wavelength because it was previously found to be very active in increasing the CAP (Compound Action Potential) of the sciatic nerve.³ The 600–900 nm region was chosen because it contains the 630 nm, 780 nm and 830 nm wavelengths which are known to be active⁴ in enhancing proliferation of various cells.

Though theoretically it always seemed that laser coherency is not required to achieve biological effects, some investigators claim⁵ that they did not get biological effects with non-coherent light sources. In recent papers^{1, 2, 6} we tried to explain light-induced proliferation. We assumed that 540 and 630 nm visible light and 700–900 nm far-red light is absorbed by mitochondrial enzymes and is converted

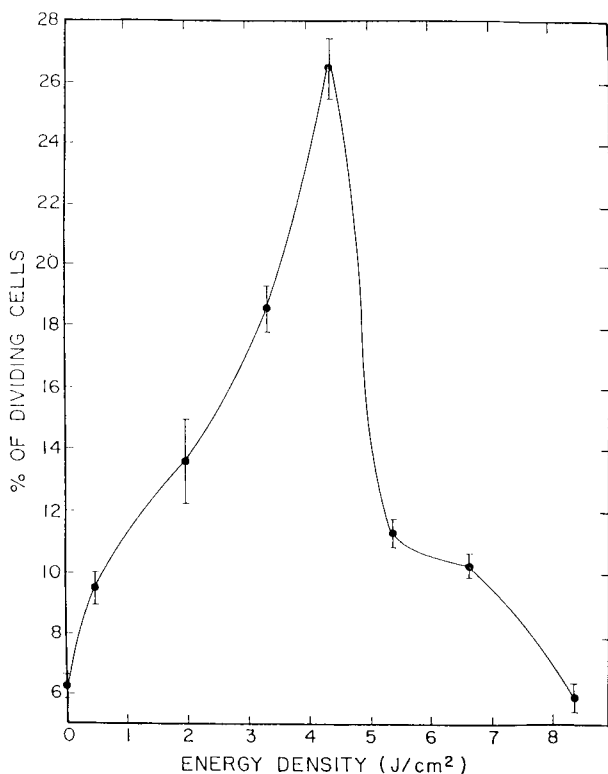


Figure 1. Percentage of dividing cells, 24 h after irradiation at 540 nm, 15 mW/cm².

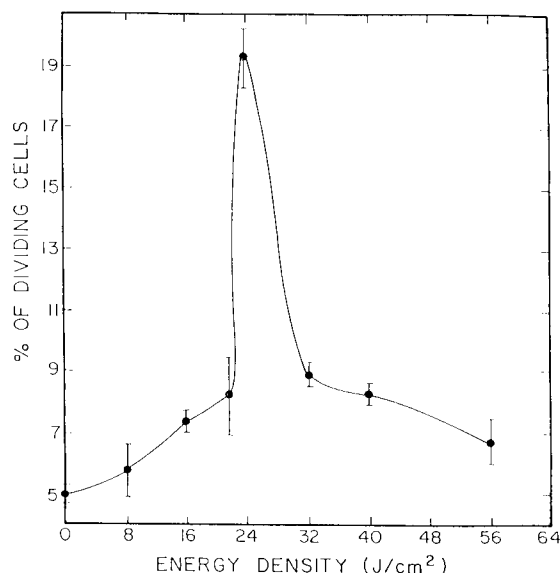


Figure 2. Percentage of dividing cells, 24 h after irradiation in the 600–900 nm region, 330 mW/cm².

to the electrochemical energy of the pmf (protein motive force).⁷ We then suggested that the increased pmf changes the Ca⁺⁺ concentration in the cytoplasm. We studied⁸ the effect of light on calcium transport and found that indeed there is an accelerated Ca⁺⁺ transport in irradiated cells. Transient changes in cytoplasmic Ca⁺⁺ concentrations can trigger cell mitosis which can explain our results.

Another important phenomena discovered in this work is that the enhanced proliferation is non-linearly intensity-dependent.

In a second series of experiments, cells were irradiated with 540 nm (Figure 3) or 600–900 nm (Figure 4), at the optimal energy dose depicted earlier and at various intensities. The resultant cell division was enhanced non-linearly with light intensity. The non-linearity is discussed in a separate paper.⁹ The shapes of the curves are very similar to those obtained by Karu¹⁰ for *E. coli* cultures. The dependence on intensity can explain the failure of some investigators to get biological effects, although they used the correct wavelength and energy doses. It is important to note here that the effect of enhanced proliferation, in fibroblastic cells, obtained in this work, is quite large, a result which may interest dermatologists.

As low energy lasers are also used for various medical applications, it is important to take the new parameter (intensity) into consideration, especially when transcutaneous irradiations are used. No effect will be obtained if the intensity of the light source will be below a certain threshold, even if the energy dose is correct.

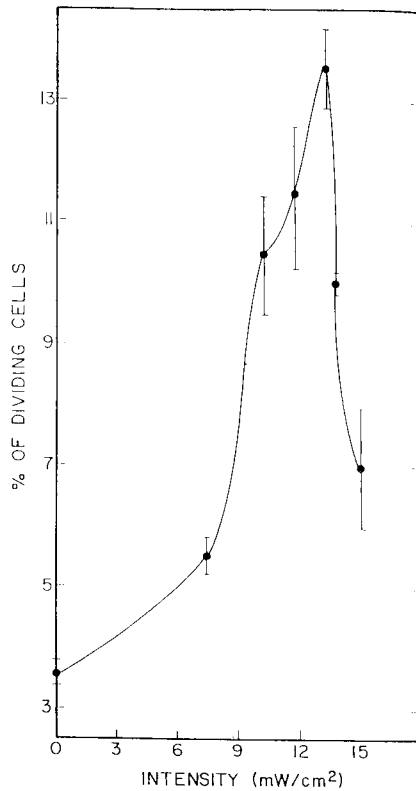


Figure 3. Percentage of dividing cells, 24 h after irradiation at 540 nm at a constant energy density of 4 J/cm².

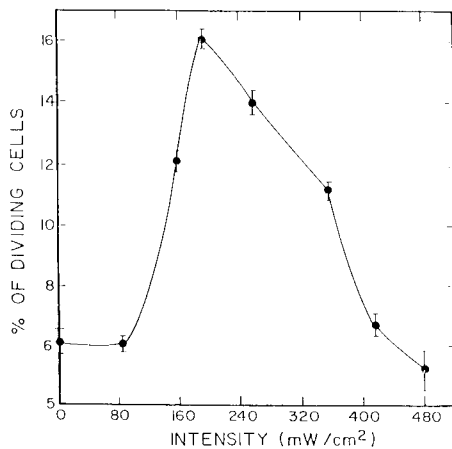


Figure 4. Percentage of dividing cells, 24 h after irradiation in the 600–900 nm region at a constant energy density of 24 J/cm².

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