Photomedicine and Laser Surgery
Volume 27, Number 3, 2009

Pp. 441–446
DOI: 10.1089/pho.2008.2285

# Effects of a Polarized Light Source (400–2000 nm) on Hep.2 and L929 Cell Lines: A Spectroscopic *in Vitro* Study

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## Abstract

***Objective:*** The aim of this study was to evaluate the effects on Hep.2 cells originating from laryngeal carcinomas, and L929 cells originating from a fibroblast line, subjected to polarized light at a wavelength of 400–2000 nm. ***Background Data:*** Recently there has been increased interest in the propagation of polarized light in randomly scattering media such as biological tissues, because of its potential applications in medicine. ***Materials and Methods:*** Irradiation was performed at two time points: T0 (24 h after cell culture) and T48 (48 h after the first irradiation). Cellular viability was assessed using an MTT assay at the following times: T0 (first irradiation), T6 (6 h after the first irradiation), T12 (12 h after the first irradiation), T24 (24 h after the first irradiation), T48 (48 h after the first irradiation), and T72 (72 h after the first irradiation). The results were analyzed using Graphpad Prism software. ***Results:*** The results showed that time influenced the cellular viability of L929 cells of both control ($p = 0.0014$) and illuminated cultures ($p = 0.0035$). Significant differences between control cells ($p = 0.0001$) and illuminated Hep.2 cells ($p = 0.0001$) were observed. There was a significant difference between the proliferation of the two types of cells illuminated compared to their controls: Hep.2 ($p = 0.0001$) and L929 ($p = 0.0002$). ***Conclusion:*** The use of polarized light on Hep.2 and L929 cells resulted in photobiological effects that need further investigation, as this is the first study using this methodology.

## Introduction

The effects of laser irradiation on different cell lines have been previously described in the literature. Our group has recently reported on the effects of different wavelengths on several cell lines, and we found that dose, wavelength, power density, and nutritional status of the cell culture all determine the magnitude of cellular proliferation.[1–3]

We also suggested that both stimulation and inhibition of the photoreceptors of the respiratory chain of the cells influence cellular proliferation.[4] It is also known that alterations in the membrane potential of the mitochondria interfere with cell metabolism. Increases in both the electric potential and consumption of oxygen by cells increases the production of both ATP and RNA, and increases protein synthesis.[3–6]

It is important to understand the effects of light stimulation on malignant cells due to the possibility of their being irradiated during clinical treatment. We have shown that irradiation at certain wavelengths significantly increases cell proliferation, and that the use of small doses and low power densities has a positive influence on cellular metabolism, and that the physiologic status of the cell also influences cell proliferation.[1,2]

Recently the effects of polarized light on tissues and cells has become an area of interest for researchers due to its wide variety of applications, particularly in biomedicine. Recent studies have shown that illumination by polarized light sources may result in similar biological responses to those seen with the use of laser light. This technology is easy to handle, has low cost, and its use may be come widespread in the future.[7,8]

Several previous reports on the use of polarized light sources showed some interesting biological properties, such as increases in the activity of cellular membranes, stimulation of regenerative processes, and increased formation of ATP. It has also been shown to have positive effects by speeding healing, reducing exudation, increasing the deposition and organization of collagen fibers, and hastening re-epithelialization. It also increases tissue oxygenation by in-

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creasing vasodilatation, and increases the number of myofibroblasts.[5,6,9–11]

Studies of wound healing have shown that smaller numbers of myofibroblasts result in less wound contraction during wound healing,[12] and that larger numbers of myofibroblasts result in more wound contracture, which is undesirable in most situations. However, wound contracture may favor the healing process of large ulcerations and extensive burns.[12]

Polarized light affects the lipid layer of the cellular membrane, as the polarized ends of lipids tend to rotate towards the light source, changing their structure. Transference of energy from the lipids to proteins and the resulting reorganization of the cellular membrane occurs due to this closer contact.[13,14] These alterations influence cellular processes related to the membrane of the cell, such as the production and transfer of energy, the immune response, and enzymatic reactions.[9,10]

The polarized light sources we have been working with are polychromatic, with wavelengths ranging from 400–2000 nm, within both the visible and the invisible spectrum. Due to its wide spectrum this type of light affects biological tissues due to the influence of both the visible and the invisible wavelengths acting simultaneously.[10,15,16]

Several mechanisms are responsible for the biostimulating effects of this type of polychromatic light source. These may result in the same types of cellular responses, but the two types of light affect the cascade of metabolic events at different levels.

Previous reports have shown similarity of the results seen with the use of laser light and of polarized light.[7,8] Polarized light has nearly equal effectiveness (80%) as that of helium-neon (He-Ne) laser light.[13,17] It has been suggested incoherent polarized light sources may induce biostimulating effects in living cells similar to those of low-level lasers.[13,17–19]

Some characteristics of laser light, such as its coherence and monochromaticity, are no longer considered necessary to achieve phototherapeutic effects, and the combination of visible and infrared light has become a popular phototherapeutic tool. Because of this fact, it is valuable to assess the effects of such a device, as this technology is easy to handle and has low cost.

The aim of the present investigation was to compare, through the use of the MTT assay, the effects polarized light system (400–2000 nm) on cellular cultures of human laryngeal carcinoma cells (Hep.2), and cells of fibroblast lineage (L929-CLLINCTC clone 929, or L929).

Table 1. Mean Absorbance (Arbitrary Units ± SD) of Illuminated and Non-Illuminated L929 Cells in Culture

| *Time (h)* | *Polarized light* | *Control* |
|---|---|---|
| 0 | 0.79 ± 0.12 | 0.76 ± 0.23 |
| 6 | 0.63 ± 0.14 | 0.50 ± 0.13 |
| 12 | 0.88 ± 0.14 | 0.89 ± 0.06 |
| 24 | 0.71 ± 0.29 | 0.87 ± 0.21 |
| 48 | 0.97 ± 0.08 | 0.84 ± 0.28 |
| 72 | 1.04 ± 0.09 | 1.07 ± 0.07 |

Table 2. Mean Absorbance (Arbitrary Units ± SD) of Illuminated and Non-Illuminated Hep.2 Cells in Culture

| *Time (h)* | *Polarized light* | *Control* |
|---|---|---|
| 0 | 0.50 ± 0.08 | 0.45 ± 0.13 |
| 6 | 0.29 ± 0.03 | 0.31 ± 0.03 |
| 12 | 0.65 ± 0.07 | 0.91 ± 0.08 |
| 24 | 0.63 ± 0.09 | 0.48 ± 0.15 |
| 48 | 0.71 ± 0.15 | 0.62 ± 0.16 |
| 72 | 0.71 ± 0.06 | 0.64 ± 0.13 |

## Materials and Methods

L929-CLLINCTC Clone 929 (fibrosarcoma fibroblasts) and Hep.2 (human laryngeal carcinoma) cells acquired from the Bank of Cells of the Federal University of Rio de Janeiro were maintained frozen in flasks at –190°C. The cultures were kept in MEM medium (minimum essential medium; Invitrogen, São Paulo, Brazil) with 10% BFS (bovine fetal serum), 1% L-glutamine, and a 1% antibiotic solution (250 $\mu$g/mL streptomycin and 80 mg/mL gentamicin sulfate; Gibco). A concentration of 20% of BFS was used after defrosting and 10% $NaHCO_3$ was added to the medium to maintain the pH around 7.2. The cells were incubated in an atmosphere of 5% of $CO_2$ at 37°C. Following trypsinization, aliquots of the culture ($5 \times 10^4$ cells/mL) were placed on 96-well plates and incubated overnight in MEM with 10% BFS in a 5% $CO_2$ atmosphere at 37°C.

In order to prevent any interference during the experiment a well was left empty on each plate between the experimental wells. Each plate had six samples. The culture medium was changed at 48-h intervals. Before the experiment was begun all cultures were examined under light microscopy and viability of the cultures was confirmed three times using the tryptan exclusion test.

Twenty-four hours before irradiation, 50 $\mu$L of a cell suspension ($2 \times 10^4$ cells/mL) was placed in each of 24 wells of the 96-well plates containing MEM medium (10% BFS, 1% L-glutamine, and a 1% antibiotic solution). The suspensions were then irradiated for 2 min with a polarized light source (Bioptron®; AG Suisse, Wollweack Switzerland; 400–2000 nm, 40 mW, 2.4 J/cm$^2$/min). The fluence was 4.8 J/cm$^2$, resulting in a total dose of 9.6 J/cm$^2$. The irradiations were carried out for 48 h total (at time points 0 and 48 h), and the spot size was adjusted to fit the diameter of the well. Cellular viability was determined using the MTT assay.[20]

The viability assay calculated the number of cells with the equation: viability = number of living cells × 100/number of dead cells. A spectrophotometer (570 nm; Spectra Count, Packard Instruments, Meridan, CT) was used to determine the optical density (OD) of the irradiated and control cells at 0, 6, 12, 24, 48, and 72 h after the first irradiation. The results were statistically analyzed using GraphPad Prism® software. (Graphpad Software, La Jolla, CA).

## Results

The results of the mean absorbance found on the MTT assay can be seen in Tables 1 and 2. The growth curves of both

the control and experimental groups can be seen in Figs. 1, 2, 3, and 4.

The normality of the data was verified and due to the small sample size we used the non-parametric Kruskal-Wallis test and Dunn's multiple comparisons test, with significance set at $p < 0.05$. The results showed that time had a significant influence on both control ($p = 0.0014$) and irradiated ($p = 0.0035$) L929 cells, and on control ($p = 0.0001$) and irradiated ($p = 0.0001$) Hep.2 cells. The influence of the treatment on the viability of cultures of both cell lineages was also significant compared to their controls (Hep.2, $p = 0.0001$; L929, $p = 0.0002$).

## Discussion

Research using cells in culture is now widely used in biomedical research due to restrictions on animal experimentation in several countries. There are several previous reports on the effects of phototherapy on different cell lineages.[1-4,6,21,22] Even so, controversy remains with regard to the effects of light sources on different tissues, especially on cell proliferation.[1,2,3,5,22,26] It is known that photobiomodulation has beneficial effects on living tissues.[4,5,25] Several studies of cells in culture have used the MTT assay[24] to assess cellular proliferation.[1,2,3]

A previous report using fibroblasts in culture showed that lineages kept under nutritional deficit, grown in medium supplemented by only 5% FBS, had a rate of cell proliferation significantly smaller than that of cells grown under ideal culture conditions (10% BFS). It was also shown that when irradiated, cells kept under nutritional deficit had cell growth rates similar to or higher than those of control cells kept under ideal culture conditions. This report suggested that laser energy had a more prominent effect on cell cultures under nutritional stress. Different BFS concentrations were used and the results showed that cells cultured in serum-free medium had no growth, while cells grown in medium containing only 5% BFS had a growth rate significantly smaller than that of cells grown in an ideal serum concentration (10%).[27]

Previous reports from our group have also shown that nutritional stress may induce neoplastic cells to absorb more laser energy due to their intense metabolic activity, as malignant tumors are constantly nutritionally deficient. This is an important factor, because previous reports by our team and the results of the current investigation show that nutritional stress may result in higher proliferative activity of cancer cells,[1–3] and that cells in culture, independent of the effect of laser irradiation, follow their normal life cycle. In our study we used cell cultures under ideal culture conditions (10% BFS), as cells grown under ideal conditions do not respond to light energy in the same way as cells at a nutritional deficit.

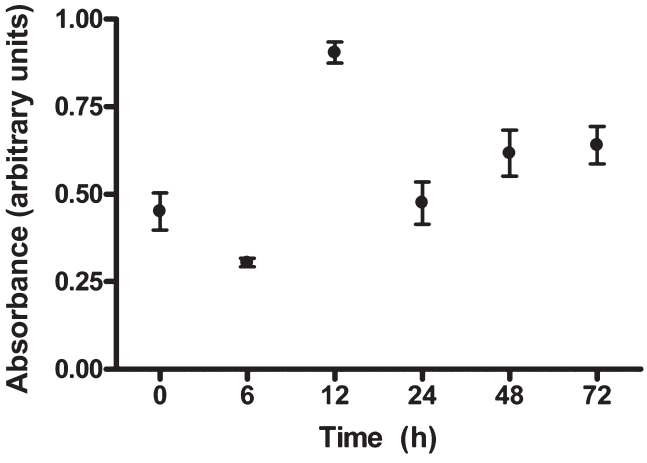


**FIG. 1.** Growth curve of control Hep.2 cells.

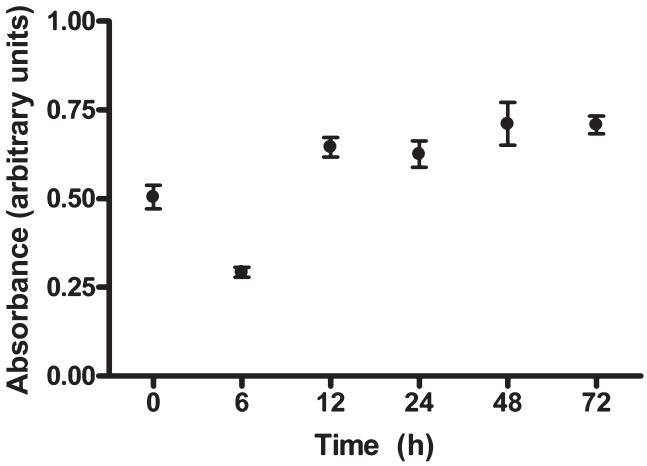


**FIG. 2.** Growth curve of irradiated Hep.2 cells.

In this study, factors such as temperature, humidity, and lighting, which may interfere with cell growth, were consistent for all study groups. However, other unknown environmental factors may have influenced cell growth.

Polarized light vibrates in a single direction, perpendicular to its propagation axis. This characteristic allows it to act on the lipid layer of the cell, altering cellular processes regulated by the cellular membrane such as energy production, ion transport, and immune processes.[28]

It has been suggested that that coherence, monochromaticity, and polarization are key properties responsible for the effects of laser photobiomodulation (LPBM). But some studies suggest that laser coherence and monochromaticity may play a less important role in photobiomodulation than the polarization of light, and that the combination of visible and infrared (IR) wavelengths produces a more pronounced effect than using either visible light or IR alone.[29]

Alternative light sources have been used to accelerate wound healing, and previous reports suggest that polarization is the characteristic of laser energy most responsible for its biomodulating effects, and thus other types of polarized light sources may also have biomodulating effects.[28,30,31]

Polarized light is able to induce biostimulative effects in living cells similar to those of LPBM. As the Bioptron lamp combines visible light at 480–700 nm and IR light at 700–2000 nm, it is a low-power light source similar to a low-level laser, but its light is polychromatic and incoherent. One of the main effects of the absorption of visible light is the stimulation of mitochondria, which results in increased cellular energy and activation of nucleic acid synthesis, both of which are essential for wound repair.[6]

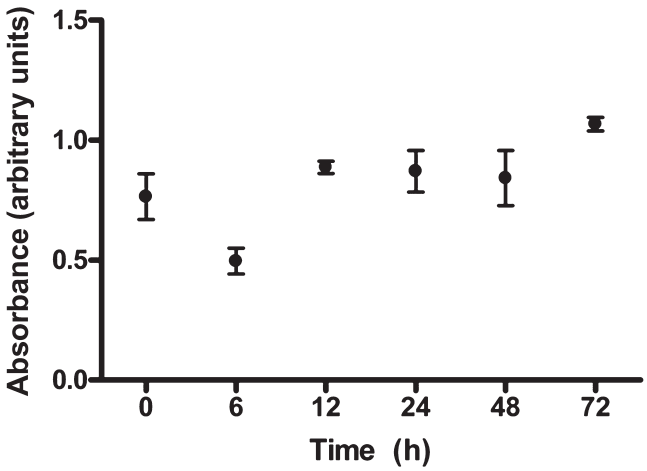


**FIG. 3.** Growth curve of control L929 cells.

Polarized light may reproduce nearly 80% of the effects of the LPBM, but non-polarized light may not. Polarization has also been suggested as an important factor in the tissue response to irradiation.[28] As reported in the literature, linear polarized light affects cellular processes regulated by the cell membrane, including energy production, ion transport, and immune processes.[32] These effects directly influence tissue repair, particularly in cases of homeostatic imbalance.[33]

Hep.2 cells have been used for biological studies over the last 50 years, supplying important data that help us understand the behavior and proliferation of neoplastic cells in culture.[1–3] Similarly several studies have been carried out using different fibroblast cell lineages. We chose to use L929CLLINCTC clone 929 cells that have been used previously in several studies involving cell viability assessment.[6,22]

A previous report from our group showed that laser energy has similar absorption by both neoplastic and non-neoplastic cells in culture, and that both can be stimulated depending on the irradiation parameters used. Our group found that laser energy (685 nm) is capable of inducing faster cellular responses than IR laser energy (830 nm), and this may be attributed to the different parts of the cell activated by the two types of light energy.[3] We have also found that nutritional stress may increase proliferation of cancer cells irradiated with laser energy. It is also important to remember that cancer cells have high rates of metabolic activity and are under constant nutritional stress. That is an important factor, as previous studies have shown that nutritional stress associated with phototherapy increases cancer cell proliferation.[1,2,3,23]

We carried a study with Hep.2 cells using LPBM (685 or 830 nm at 4 J/cm$^2$) to verify the influences of time and wavelength on cellular viability of this cell line using the MTT assay.[3] In that study we found that time has a significant effect on cellular proliferation of cells irradiated at 685 nm, but not at 830 nm. This was attributed to the specificity and localization of the chromophore activated by each wavelength. In the present study we found a different result, as irradiated Hep.2 cells showed an increase in cellular viability up to 12 h. However, after the second irradiation (48 h later) there was no increase in cellular proliferation in either group. After 48 h, the cultures maintained their standard cellular cycle. This finding corroborated those of other studies also using Hep.2 cells in culture, that were irradiated at 670 nm (0.04–4.8 J/cm$^2$).[1,2]

Recent studies have concluded that both dose and wavelength are factors that may influence the proliferation of these cells, and these results corroborate the findings detailed here. In the present study comparison of cellular viability of non-irradiated and irradiated Hep.2 cell cultures showed that the treatment significantly influenced cellular viability. On the other hand, in control cultures only time had an influence on cellular proliferation.

Other lineages of neoplastic cells have been used by researchers with the objective of verifying increases in cellular proliferation due to LPBM. We also have used oral carcinoma cells (KB lineage) as a model to assess the effects of photobiomodulation,[23] and also found that cellular viability of irradiated cells was significantly higher than that of controls; in other words the treatment with the laser had an influence on proliferation, similarly to the findings presented here. Another study carried out in Russia observed exponential growth of He-La cells following exposure to laser light (632.8 nm and 100 J/cm$^2$).[34] That study showed that during the first 4 h after irradiation, there was an increase in the number of cells, which was followed by a decrease to levels similar to those of non-irradiated cells. This was not found in the present study, as we found decreases in cellular proliferation in Hep.2 cells in both groups (irradiated and non-irradiated) in the first 6 h, which was followed by an increase in the next 6 h. This difference may be due to the differences in the applied doses.

A previous report studied cellular proliferation using gingival fibroblasts in cultures irradiated with laser energy (670 or 780 nm and 2 J/cm$^2$). It was found that both wavelengths were capable of stimulating the proliferation of fibroblasts. These results are similar to those detailed here, as L929 cells showed increased proliferation at a low dose and with wavelengths within both spectra.[27]

Previous reports on the use of polarized light to affect proliferation of fibroblasts have shown that proliferation is more

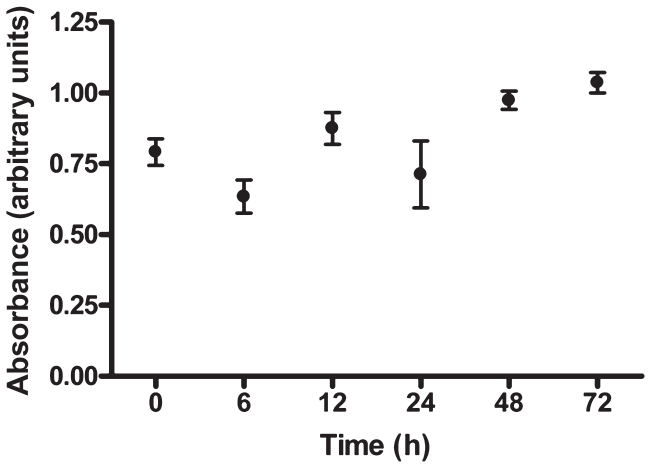


**FIG. 4.** Growth curve of irradiated L929 cells.

intense 24 h after irradiation, and that later on it begins to decrease.[6,22] This finding corroborates our findings, as we showed that polarized light also increased the proliferation of L929 cells after 24 h. The comparison of the cellular viability of irradiated and non-irradiated L929 cultures showed that the treatment had an influence on cellular viability, and that time was significant in both groups.

## Conclusion

We conclude that the use of polarized light on both malignant and non-malignant cells in culture resulted in increased proliferation as determined by the MTT assay, and we believe that the photobiological effects of polarized light require further investigation.

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