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Impacts of various intensity red lasers at 635 nm on colon cancer cells

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Abstract
With low-level laser treatment, this study aims to determine whether different intensities may promote prevent or accelerate cell growth. Different power densities were used to irradiate colon cancer cells. Laser irradiation at 5 J/cm^2 with a wavelength of λ-635 nm was done continuously. The experimental groups were designed to achieve the following results: The control group was the non-irradiated group, and the irradiated experimental group was as follows: the first group the power of 70 mW and intensity of 61.94 mW/cm^2 was the second; 90 mW and 79.64 mW/cm^2 were the third; 110 mW and 97.3 mW/cm^2 were the fourth; and 130 mW and 115 mW/cm^2 were the fifth group. Changes in cell proliferation have been examined using MTT assays. The findings showed that different power densities enabled noticeable alterations in the cells. They concluded that at a certain power density, cells react optimally.

Keywords: MTT, colon cancer cells, low-level laser

1. Introduction
Utilizing the phenomena of stimulated emission, a laser generates and amplifies electromagnetic radiation at a certain wavelength [1]. A laser is a device that produces a strong, parallel, monochromatic beam [2]. Laser light has an extremely clean frequency, which makes it excellent for biological applications [3]. Rather than the kind of tissue exposed, laser beams are categorized as hard or soft tissue lasers depending on how the laser interacts with the tissue. Physical therapy employs cold laser treatment, a type of laser medicine that modifies biological processes by using light-emitting diodes or low-level lasers. The characteristics of laser irradiation, such as exposure time, wavelength, laser output power, and fluence, are critical to the *in vitro* biostimulation achieved by LLLT. Protein synthesis can be hampered by conditions that successfully increase cell proliferation [4]. To eventually achieve the intended effects on patients, it is imperative to understand the proper mix of characteristics (case in point, wavelength, power density, and energy density) [5]. Enhancing cell growth may also need consideration of power density. The purpose of this work is to examine how the biomodulation of colon cancer cells is affected by varying low-power diode laser power densities (mW/cm^2).

2. Equipment and Techniques
2.1 Culture of cells
Since cells may be coaxed to develop in the laboratory or their tissue in appropriate conditions when given a medium containing nutrients and growth hormones, cell cultivation is among the widely extensively used procedures in medical studies. In a lab culture setting, cells are paired with the vivo and allowed to grow similarly to the organism. At 37 °C, the cell cultures were being brooded. Inverse microscopy was employed to examine the cells [6]. The complicated media encourages these cells to proliferate in handy plastic tubes.

2.2 Laser irradiation
Red lasers with wavelengths of 635 nm, power densities of 61, 94, 79.64, 97.3, and 115 mW/cm^2, and dosages of 5 J/cm^2 were used to irradiate colon cells. Three irradiation tests were conducted in triplicate to determine how these intensities affected the cells, and the median value of the findings was taken.

To help the cells stick together, they are incubated for the whole night after seeding. Before being exposed to radiation, the plates were cultured at 37 °C [6]. To see whether there were any notable alterations in the cells during the laser irradiation, the cells were also maintained at *vitro* temperature.

2.3 MTT test

The MTT test tries to compute cell viability in a high-resolution method, eliminating the need for repetitive cell count. Because mitochondrial succinate dehydrogenase can only degrade solubility tetrazolium salts into a darker purple formazan crystal in active cells (Figure 1), the degree of activation is a good indicator of the potential quantity of cell division. The MTT test is predicated on the idea that most viable cells have a stable mitochondrial function and that changes in the number of viable cells are directly correlated with changes in mitochondrial activity. utilizing a plate reader calibrated at 570 nm, formazan concentration represented in optical density is measured to assess any increase or reduction in cell viability [7].

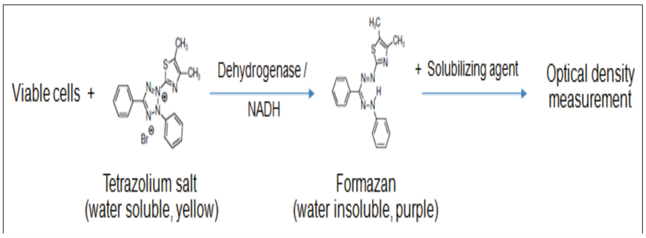

Fig 1: Reduction of the formazan in living cells from the MTT tetrazolium salt [8].

3. Lower-Level Laser Therapy (LLLT)

A laser is a device that releases light by the emission of photons through an optic amplification process. The 1960s saw the discovery of LLLT, a type of complementary therapy [9]. To encourage healing, modest energy densities of red or near-infrared light are applied to cells. LLLT is sometimes known as "cold laser therapy" because of the low power densities that are utilized to avoid heating the tissue [10].

3.1 The Low-Level Laser Therapy History

Townes' discovery that regular light might be altered to produce brief waves led to the discovery of lasers in the early 1960s. On the other hand, Arthur Schawlow invented the method for efficiently emitting light waves. He discovered that to allow the waves to radiate, it would be ideal to contain the atoms in a long, narrow chamber with mirrors fastened at either end. To produce light, Gorden Gould looked into techniques to "pump" atoms into higher energy states. This research contributed to the invention of the laser. The term "Laser," which stands for "Light Amplification by Stimulated Emission of Radiation," was created by Gould. The three scientists investigated the advantages of using different gasses and crystals to boost atom energy in more detail [11]. Following the introduction of the ruby laser in 1960 and the helium-neon laser in 1961, low-level lasers were used. 18 The advantages of laser therapy were initially noted by Endre Mester. He noticed this when he used a Helium-Neon (He Ne) laser to treat hairless mice, which caused the animals to start growing hair. Mester treated patients with non-healing skin lesions after making this finding [10].

3.2 Light-producing sources

Nowadays, LLLT uses light from a variety of sources. In addition to gallium arsenide semiconductor diode lasers, light-emitting diodes (LEDs) are presently becoming more and more common laser light sources. He-Ne, a gas that produces light with a wavelength of 632.8nm, is another frequently utilized light source in lasers. Light-emitting diodes (LEDs) are becoming more and more popular since they can emit light at a far broader spectrum of wavelengths than any other laser possible. Organic LEDs with an electroluminescent layer that generates light in response to an electrical current are the newest light sources being studied as new technology becomes accessible [12].

3.3 Mechanisms

Through a mechanism called photobiomodulation, LLLT is thought to cause photochemical reactions in the cells [10]. Tissues are impacted by LLLT because of a phenomena where light reflection modifies the refractive indices of air and tissue. The law of Snellius may be used to summarize this [10]

$$\frac{\sin\theta_1}{\sin\theta_2} = \frac{n_2}{n_1}$$

The angles represented by θ1 and θ2 is between the normal air surface and light, the normal tissue surface and laser beam, and the air and tissue refraction indices, respectively, are n1 and n2. According to this computation, light in tissues exhibits a "scattering behavior" that modifies the volume distribution and light intensity in tissues [10]. LLLT makes use of fluence, or the energy level per unit area of a specific size, as well as visible (380-700 nm) and infrared light (700-1000 nm). Joules per centimeter squared is the unit of measurement for fluency. The power density, measured in Watts per centimeter squared, is an additional parameter for lasers [13]. Red and near-infrared light wavelengths are among those that are commonly employed in LLLT [10]. It has recently been determined that LLLT has an impact on the mitochondria of cells, which leads to an increase in reactive oxygen species, a decrease in adenosine triphosphate (ATP), and a stimulation of transcription factors [10, 14]. A photon of light is absorbed by chromophores when a laser is administered to cells. An electron will move from a low-energy orbit to a high-energy orbit when the photon is absorbed. Numerous cellular processes are triggered by this electron flow [10].

4. Results

Laser irradiation at 635 nm and 5 j/cm^2 was utilized in this experiment. There were five groups total: one was a control group (unirradiated), and the other four were treatment groups that received laser radiation. By employing 635 nm at different power densities, the MTT test demonstrated a considerable inhibit and strengthened feasibility. This study discloses improvement in the ability of cells to grow at a power of 115 mW if compared to the non-irradiated control group, while there was a clear inhibition in the growth of the treated cells compared to the control group which witnessed the highest inhibition in cell growth when 61.94, 97.3, and

79.64 mW/cm^2 power densities respectively (Fig. 2), indicating that irradiated colon cells have attenuated mitochondrial function as compared to the untreated group.

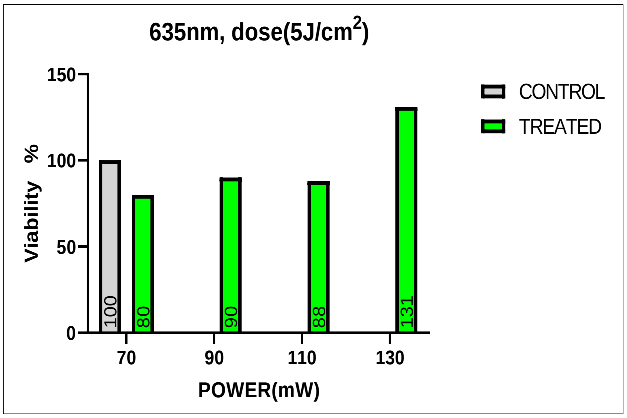

Fig 2: Impact of different red laser low-level power densities

5. Discussion

Many studies have documented the positive effect of low-level laser treatment [15-18], while many researchers have proven the harmful effect or the absence of any positive effect of cold laser treatment [19-21]. These contradictory results may have negative implications due to wide variation in individual studies of the laser parameters used. In addition, the mechanism of action and optimal parameters for laser treatment are still unclear. It was discovered in this research that the growth of cultivated cells at the same energy density may be affected differently by soft laser treatment. At 635 nm, the irradiated groups had decreased or suppressed growth rates compared to the control groups, especially at intensities of 61.94, 79.64, and 97.3 mW/cm^2. However, at a power density of 115 mW/cm^2, there was an increase in cell development. This is because low-power laser radiation may stimulate cells. Power density is generally more important in cell proliferation than the overall dosage, according to a search [22]. Therefore, *in vitro* cell proliferation research will require the measurement of different power densities. In order to ascertain the optimal laser irradiation parameters for enhancing cell development, they examined the effects of intensity modifications while maintaining the same dose (5 J/cm^2). Perhaps the type of cell or tissue targeted plays a prominent role in the biological responses, whether the effects are negative or positive. Each cell may also have its ideal irradiation parameters, and this is what was observed in this research paper and was observed in previous studies [14]. The findings of this study revealed that an increase in power affects the photochemical response or cellular activity generated by laser light, maybe due to a minor rise in the temperature of the cell's target. Changes in intensity may lead to a change in the biological behavior of the treated cell that may cause this inhibition or/and decrease in viability. Because biostimulatory or inhibition effects are closely related to the absorption of light by specific photoacceptors, located inside the mitochondria. Moreover, the survival of cultivated cells outside the incubator during prolonged irradiation periods may negatively or positively affect the growth and reproduction of cells. Therefore, low-intensity laser treatment remains shrouded in a kind of mystery based on the results and facts reached by researchers. According to the data obtained, it is not possible to predict the biological behavior of cells exposed to radiation due to many variables (cell type, culture conditions, laser type, intensity, irradiation time and doses used etc.) and this is part of the many laser parameters that play a major role in causing this behavioral changes in the cell, whether they are inhibitory, repressive, or stimulatory.

6. Conclusion

One of the key factors influencing behavioral changes in cells is varying power densities. Applying a 5 J/cm^2 dose of a 635 nm red laser with different power densities had both beneficial and harmful effects on the cells. 61.94 and 115 mW/cm^2 were the recorded maximum impacts.

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