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Keywords:	Low Level Laser Therapy (LLLT), cell proliferation, DNA damage, DNA repair, Light Emitting Diodes (LEDs)

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Assessing the Impact of Low Level Laser Therapy (LLLT) on Biological Systems: A Review

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Running Title: Impact of LLLT on Biological Systems

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Abstract

Purpose: Low level Laser Therapy (LLLT) in the visible to near infrared spectral band (390 – 1100 nm) is absorption of laser light at the electronic level, without generation of heat. It may be applied in a wide range of treatments including wound healing, inflammation and pain reduction. Despite its potential beneficial impacts, the use of lasers for therapeutic purposes still remains controversial in mainstream medicine. Whilst taking into account the physical characteristics of different qualities of lasers, this review aims to provide a comprehensive account of the current literature available in the field pertaining to their potential impact at cellular and molecular levels elucidating mechanistic interactions in different mammalian models. The review also aims to focus on the integral approach of the optimal characteristics of LLLT that suit a biological system target to produce the beneficial effect at the cellular and molecular levels.

Methods: Recent research papers were reviewed that explored the interaction of lasers (coherent sources) and LEDs (incoherent sources) at the molecular and cellular levels.

Results: It is envisaged that underlying mechanisms of beneficial impact of lasers to patients involves biological processes at the cellular and molecular levels. The biological impact or effects of LLLT at the cellular and molecular level could include cellular viability, proliferation rate, as well as DNA integrity and the repair of damaged DNA. This review summarises the available information in the literature pertaining to cellular and molecular effects of lasers.

Conclusions: It is suggested that a change in approach is required to understand how to exploit the potential therapeutic modality of lasers whilst minimising its possible detrimental effects.

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Key words: Low Level Laser Therapy (LLLT); cell proliferation; DNA damage; DNA repair, Light Emitting Diodes (LEDs);

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1. Introduction

Laser therapy or low-level laser therapy (LLLT) has been widely used for over 50 years [1]. Evolutionary, it emerged in its modern form after the invention of the laser in 1960 becoming a widespread treatment modality in a variety of clinical applications [2-4]. Investigators introduced a diverse set of terms to describe this potentially beneficial treatment tool [5]. Initially, expressions such as 'photobioactivation' and 'biostimulation' frequently relative to the stimulation effect of low level lasers were used [6, 7]. Subsequently an inhibitory effect of this radiation were also noted, which led them to coin the term 'biomodulation' [8].

Recently a consensus decision was taken to use the terminology "photobiomodulation" or "PBM". Where some researchers gave LLLT a status of subjectivity, and it is limited for actual laser specific interactions, this is not a requirement for in-coherent light emitting diodes (LEDs) which can work equally well [9]. On the contrary, other researchers reported that although LLLT is a well-established researchable, and for much time used by clinician and researchers, but it is not optimal. It is a broad term that could include photodynamic therapy (PDT) and optogenetics, these techniques use lasers and LEDs with low dose and require exogenous chromophores, unlike LLLT that utilise endogenous chromophores with low dose of light delivered at the target site. However, they also suggest using photobiomodulation (PBM), since it is more ideal, has specific definition for this application of light to be more accurate and can confirm its scientific principle [10]. Specialists of medical field successfully used photobiomodulation in treating many health conditions when other methods had had limited success, such as healing-resistant wound, chronic diabetic ulcers, injuries of spinal cord and nervous system and pain management [11]. Nevertheless, photobiomodulation is not considered as a part of mainstream medicine as still not standard treatment [12].

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LLLT treatment has evolved over the years and is being developed as a sophisticated tool for therapeutic procedures and utilized clinically for several different ailments [13]. The therapeutic treatments are based upon three principles; to minimize inflammation, edema, and chronic disorders of joints by targeting brain, skin, joint etc. [14], to promote wound healing of superficial and deeper tissues, neurological damage etc. [4, 15], and to treat neurological disorders and pain [13]. Recently, many studies on PBM therapy at infrared IR wavelengths, in particular from 700 nm up to the near infrared NIR [16-18], which was shown to produce more benefit impacts than red light in many medical conditions, including neural stimulation (by triggering direct activation of neural tissue) [19], photoaging (where IR radiation evidently has a biphasic effect), anti-tumor action (IR radiation is capable of inhibiting the proliferation of cancer cells and enhances chemotherapy efficacy, and brain neuroprotection (treatments for stroke, Traumatic brain injury (TBI) in vivo models [19, 20], and neurodegenerative disorders for Alzheimer's and Parkinson's diseases. These are given, in addition for many other diseases in Table1. Therefore, a better understanding of the mechanisms using IR radiation, could support improved therapeutic effectiveness via new strategies of PBM therapy at IR wavelengths [21].

Laser is a device which produces intense, monochromatic, coherent, and highly collimated beam of light [22]. Laser light has quite pure frequency, which makes it useful for biomedical applications [23]. Laser therapy involves visible red and near infrared (NIR) portions of the electromagnetic spectrum (390–1600 nm and 10^{13}–10^{15} HZ) , because researchers have shown that these portions of the spectrum have been absorbed highly by the biological systems and bring about a beneficial therapeutic effects in living tissues [24]. According to the portion of the spectrum (wavelength) that strikes the tissue and the intensity (power density or irradiance) of laser radiation, the photobiological impacts of laser therapy on tissue are different that lead to divide the laser therapy into two classes [25]. Class I, which refers to

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radiation of wavelengths ranges (<390 nm) and (>10,600 nm) and high power and intensity levels, are used for ablation, cutting and sterilization, because of its thermal effect. Class II, which refers to radiation of wavelengths ranges (390 -10,600 nm), levels of power (10^{-3} to 10^{-1} W) and intensity (10^{-1} to 10^{0} W/cm^{2}) and a dose of 10^{-2} to 10^{2} J/cm^{2} [4].

Whereas there is some agreement on the best wavelengths of light and appropriate dosages to be used (irradiance and fluence), there is no agreement on the emission mode of laser light; whether continuous wave (CW) or pulsed light is more suitable for the various applications of PBM. However, pulsed lasers in PBM therapy are used widely in clinical research [22, 26] and for medical treatment [27-30] Two types of pulsed laser are used for PBM therapy, a super-pulsing gallium-arsenide (GaAs) diode laser, which has a wavelength in the region of 904 nm and pulse duration in the range of 100–200 ns, and the semiconductor super-pulsing indium-gallium-arsenide (In-Ga-As) diode laser, which emits light at a similar wavelength (904-905 nm), producing very short pulses of light (200 ns) in the range of kilohertz (kHz) frequencies [31]. Therapeutically, the super-pulsed GaAs and In-Ga-As lasers are capable of deep penetration without the undesirable influences associated with continues wave lasers (CW) (such as thermal damage), as well as allowing for shorter treatment periods. Pulsed lasers offer potential benefits, attributed to the pulse OFF times (pulse quench intervals) following the pulse ON times, so that pulsed lasers can deliver less tissue heating.

Low intensity laser radiation is clinically well accepted tool in medicine and dentistry [32];Table 2]. It is featured by its ability to incite a thermic, non-damaging photobiological action [33]. Unlike hard high power laser, LLLT provides low energy only sufficient to induce stimulation response of body tissue, and has a wavelength-dependent manner able to change the cellular function. in the absence of considerable heating [34]. Hence, LLLT is also called soft laser therapy or cold laser, as low energy laser has no thermal effects [13, 35].

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Table1. Review of published studies using LLLT to treat different diseases.

It was observed that the broad range of laser therapy included molecular, cellular and tissue level effects and the modes of action of LLLT may vary with different confounding factors and applications [13]. To produce photo-biological action, photon absorption of laser radiation must occur [24]. Endogenous or exogenous chromophores are the initial photoacceptor molecules (i.e. molecules that can absorb light at certain wavelengths) absorb the incident photon energy [36]. A photochemical conversion of the photon energy absorbed by a photoacceptor has been demonstrated [37]. The absorbed energy of photon can be transferred to another molecule, which can then cause chemical reaction without alteration in temperature in the surrounding tissue [37, 38]. Some native component can be activated in the irradiated cell at certain wavelength, and consequently, biochemical reaction as well as cellular metabolism might be altered [39].

Several studies suggested that mitochondria is the most sensitive component of cell to visible and near infrared light [39], [40], that result in increased production of adenosine triphosphate (ATP), increased deoxyribonucleic acid (DNA) synthesis, modulation of reactive oxygen species (ROS), nitric oxygen species (NOS) and the induction of transcription factors [41]. Moreover, PBM at red and NIR wavelengths stimulate increasing intracellular calcium Ca^{2+} [42-45], however recent studies emphasised that blue (420 nm) and green (540 nm) lights are more effective in increasing Ca^{2+} when applied at the same doses [46]. Many researchers suggested that the response of some cells to blue or green light interacting by light-gated ion channels, which enable light to control electrical excitability, intracellular acidity, calcium influx and other cellular processes [47-49]. The most likely ion channel is light-gated channel rhodopsin, because the action spectra of the channel rhodopsin family displays peaks in the blue-green spectral region [50]. The precise mechanism of laser-

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tissue interaction has not been completely explained, therefore there is no ability to offer a clinical treatment protocol at present [51].

The review of the available literature suggests that the variety of studies have been mostly in vitro, using a range of cell lines for different types of LLLT and varying some of their parameters, as summarised in Table 3. It is possible to select wavelength, power density, laser beam intensity profile, polarisation and exposure time. The available information suggests both positive and negative outcomes with respect to different parameters (Table 2).

It could be concluded that conflicting results have been published which may be attributed to a disparity in study design, including the use of different laser wavelengths, and numerous illumination parameters, in addition to different confounding factors which influence the determination of different biological parameters.

Table 2. Parameters involved in LLLT applications.

Table 3. Review of published studies evaluating the effects of LLLT on different cell lines.

2. Optical Sources and Biological Interaction

Low level laser irradiation has been used in clinical practice causing biostimulation. A number of diseases and physical conditions are mentioned to respond to laser therapy (photobiostimulation) [52]. At the cellular and molecular level, there is still significant argument regarding the effectiveness of lasers in producing the desired practical responses [52].

To illustrate the therapeutic effects, through optical stimulation processes, we introduce here briefly the available light sources and their potential to interact at the cellular and molecular level. Currently these are not well supported by the literature.

Laser light is generated on the principle of light amplification of stimulated emission of radiation [53]. The beam energy of laser light is powerful because it is highly coherent

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(waves are all in phase), polarized, focused and monochromatic (a single wavelength). It was first used in ophthalmological field in the early 1960s, although, the basic principle of laser was proposed by Einstein as back as in 1917 [53]. Lasers are commonly designated and named by the type of lasing material employed. The laser medium can be a solid state semiconductor, a gas, a liquid or a solid, as in Nd:YAG lasers which employ a Nd:YAG rod as the lasing medium [54] .

Laser light is characterised by its single wavelength, although some lasers, such as dye laser, can be tuned over a wide range of wavelengths [55]. Lasers are also classified according to their intensity and if they are pulsed or continuous wave (CW), in order to identify the risk of harm to the patient [56]. In the medical field, lasers are classified as high power surgical lasers and low power therapeutic lasers [57]. Non-invasive or 'soft' lasers were introduced into medicine in the 1980s, and since then, have been seen as useful light sources for medical application [53]. The wavelengths of laser radiation used, have been investigated to show their therapeutic use [58].

LLLT or photobiomodulation, is a form of phototherapy, which is designed to apply low levels of red and near- infrared light with wavelengths in the region of 390-10600 nm and output powers up to 500 mW [59]. LLLT is effective in a number of clinical situations where the wavelength of red and near-infrared region are effective in such therapies. However, both of these two wavelength spectra are different in their photochemical and photophysical properties [58].

LLLT refers to the use of photon energy at low levels to alter biological activity with no-thermal reactions because there is little increase in the temperature of the irradiated tissue [59]. Lasers of low level intensity are suggested to be non-toxic, non-allergic and because of their ease of application, these techniques have gained wide application in many fields of

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health care [53],Table1. Phototherapy has been found to have significant effects on a variety of pathological conditions including pain attenuation, inflammation and induction of wound healing in non-heating effects [59].

From observations, it appears that LLLT has beneficial effects at the molecular, cellular, and tissue levels [60]. It has been found that medical treatment with LLLT at various intensities has stimulatory effect on cellular processes [61]. Recently, it has been reported by several investigators that at low –levels of red or near-infrared light illumination, LLLT can prevent cell apoptosis [59, 62], stimulation of mitochondrial activity, increased cell turnover, recruitment and proliferation, modulation of the cellular metabolites [63]. It was suggested that LLLT might promote changes in the cellular redox state, playing an important role in sustaining cellular activities, and induce photobiostimlative processes [64]. In addition to the above, pre-exposure of PBM had a protective effect against many external agents such as hydrogen peroxide, H_2O_2, and UV radiation [65, 66]. There is an evolutionary standpoint confirm that NIR pre-exposure protect cells from the hazard impacts of UV exposure, and the re-exposure for NIR radiation could be important for protection maintenance [67, 68]

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3. Optical Properties of Tissues

When the laser light strikes biological tissues, part of this light is absorbed, part is reflected or scattered, and the rest transmitted. Reflection phenomenon is produced due to a change in refractive index of air and tissue. Snell's law can be used to explain this phenomenon:

$$\frac{\sin \theta_1}{\sin \theta_2} = \frac{n_2}{n_1} \quad \ldots\ldots\ldots (1)$$

Where θ_1 is the angle between the incident light and the surface normal in the air, θ_2 is the angle between the ray and the surface normal in the tissue, n_1, n_2 are the refractive index of air and tissue respectively [69].

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Most of the light is absorbed by the tissue because the energy state of molecules is quantized; therefore, photonic absorption occurs only when its energy equals the energy difference between such quantized states. Absorption is key for the desired impact on tissue healing. The magnitude of optical absorption is described in terms of the absorption coefficient μ_a, in units of cm^{-1} [70]. The depth of penetration (mean free path) into the absorbing medium is defined by the inverse, I_a [13].

The primary step for tissue interaction is scattering behaviour of light in the biological tissue, which is followed by absorption, it is also important because it determines the magnitude distribution of light intensity in the tissue. Scattering of a photon is synchronous with a change in the propagation direction without loss of energy. Analogous to absorption, scattering is expressed by the scattering coefficient μ_s (cm^{-1}) [69, 71]. The length until next scattering occurs is $1/\mu_s$ (cm). Scattering is not isotropic, having a physical property that has the same value when measured in different directions. Forward scattering prevail in biological tissue. This physical characteristic is expressed by the anisotropy factor giving absolute values for isotropic scattering ($g = 0$) to forward scattering ($g = 1$). In biological tissue, g can differ from 0.8 to 0.99, and can have a considerable role in a reduced scattering coefficient, μ_s' (cm^{-1}), which can be defined as:

$$\mu_s' = \mu_s (1- g) \ldots..(2)$$

The sum of absorption coefficient (μ_a) and scattering coefficient (μ_s) is called the total attenuation coefficient, that the beam is "attenuated" (weakened) as it passes through the medium. Attenuation coefficient of the volume of a material characterizes how easily it can be penetrated by a beam of light, in other words, the fraction of an incident beam of photons that is absorbed or scattered per unit thickness of the target absorber, μ_t (cm−1):

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$$\mu_t = \mu_s + \mu_a \ldots\ldots (3)$$

3.1 Light Distribution in Laser-Irradiated Tissue

Most of the recent evolutions in describing the transfer of light energy in tissue are based on transport theory [72] (radiative transfer), the physical phenomenon of energy transfer in the form of electromagnetic radiation. The propagation of radiation through a medium is affected by absorption, emission, and scattering processes [72, 73]. According to transport theory, the radiance $L(r, s)$ of light at position r traveling in the direction of unit vector s is reduced by absorption and scattering, but it is increased by light that is scattered from s′ direction into direction s. Radiance is a radiometric measure that refers to the amount of light that passes through or is emitted from a particular area, and drops within a given solid angle in a particular direction. Then, the transport equation which describes the light interaction is:

$$s.\ \nabla L\ (r,\ s) = -\ (\mu_a + \mu_s)\ L(r,\ s) + \mu_s \int_{4\pi} p(s,s')L(r,\ s')d\omega' \ldots\ldots (4)$$

Where $d\omega'$ is the differential solid angle in the direction s', and $p(s,s')$ is the phase function [13, 74].

Determining the distribution of light in an irradiated tissue is based on the transport equation requiring μ_s, μ_a and p. An exact solution for transport equation is often difficult, therefore several approximations have been made concerning the illustration of the radiance and phase function. The approximate calculations of distributed light in tissue are related to the type of light irradiation (diffuse or collimated) and the optical boundary conditions (matched or unmatched refractive indexes) [74].

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4. The Mechanism of Laser-Sub-cellular and Cellular Interaction

It is being suggested that the key underlying mechanism of action for most of the physiological effects attributed to LLLT is the stimulation of mitochondrial activity [63], [75]. The first law of photobiology states that photons of low power light must be absorbed by electronic absorption bands belonging to chromophores to produce significant effects on living biological systems [62]. A chromophore (or photoacceptor) is a molecule of a compound, which imparts some colour to the compound [76].

According to the theory of quantum mechanics by Max Planck (1900), light energy consists of photons or discrete packets of electromagnetic energy. The individual photon energy depends on the wavelength; therefore, the dose energy of light depends on the number of photons, their wavelength and surface area through spot-size of the laser [41].

When photons from a laser are incident on living tissue, it can be locally absorbed or could scatter. Scattered photons are reflected or transmitted [41]. Absorbed photons interact with the chromophore molecule located within the tissue. The absorption of light leads to excitation of electrons to higher energy levels. The delocalized electrons of the energized molecule which are excited rise from the ground state to an excited state [77]. This excited molecule must lose its extra energy, which must be conserved according to the first law of thermodynamics. Three possible pathways occur when LLLT is delivered into tissue.

Pathway 1: The commonest pathway that occurs is called internal conversion, the excited singlet state of chromophore transport from a higher to a lower electronic state. This transition takes place without photons emitting, known as non-radiative decay [41]. The energy of the electronically excited state is coupled to rotational and vibrational modes of the molecule. Thus, this interaction increases the kinetic energy of the molecule, such that the

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excitation energy is transformed into heat. This process would not be expected to cause chemical changes to the molecule [58].

Pathway 2: The second pathway that can occur is fluorescence. Fluorescence is re-emission of light by a substance that has absorbed light. It is a form of luminescence. The excited molecule tends to return to its stable state by emitting photons with a longer wavelength (i.e., lower energy than the absorbed photon) [77]. The resultant heat (from molecular vibrations) arises from the energy difference between the absorbed and emitted photons.

Pathway 3: The third pathway that can occur after the absorption of low level laser light by a tissue photo-acceptor representing a number of photochemical processes. Although, covalent bonds cannot be broken by low energy photons, the energy is however sufficient for electrons to go from the first excited singlet state to the triplet state of the photoacceptor through intersystem crossing. Increasing the reaction rate allows transforming such as ground state molecular oxygen (a triplet) to singlet oxygen state (reactive oxygen species). Alternatively, the long-lived triplet of the chromophore may undergo electron transfer to form a radical anion, which can transfer an electron to oxygen to form a superoxide [41].

The photochemical pathway is the separation of a non-covalent bound ligand from a binding site on a metal in an enzyme. Cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain in eukaryotic cells is the candidate enzyme for a photoacceptor (chromophore), a molecule imparts a color to a compound, mediating the transfer of electrons from cytochrome c to molecular oxygen. After absorbing red or near-infrared light, cytochrome c oxidase undergoes photochemical processes through the dissociation of binding of nitric oxide from the iron-containing and copper-containing redox centres in the enzyme [41]. There is a growing body of evidence which suggests that cytochrome c oxidase could act as a photoacceptor of light in the near-infrared spectral range

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[64]. It is also considered as the photosignal transducer in the region of visible and IR-A region [78]. This reactivity is due to four redox active metal centers: the bi-nuclear CuA, CuB, heme a, and heme a3, all of which have strong absorbency in the red to IR-A range [78-80].

Many studies on the biological influence of LLLT have compared the action spectrum, a plot of the relative effectiveness of different wavelengths of light in causing a particular biological response, and under ideal conditions it should follow the absorption spectrum of the specific molecule, and whose photochemical alteration causes the biological effect attributed to the absorption spectra. These studies have suggested cytochrome c oxidase as the primary photoacceptor (chromophores) [77, 81].

Cytochrome c oxidase is the fourth enzyme in the inner membrane of cellular mitochondria [63, 82], as shown in (Figure 1), that plays a pivotal role in Adenosine tri phosphate (ATP) synthesis [64]. Excitation of cytochrome c oxidase components with infrared light energy accelerates the rate of electron transfer and in turn increases the ability of mitochondria to produce ATP, which accelerates cellular metabolic processes [64]. Moreover, signal transduction to other parts of the cell has occurred, including cell membranes [83]. Photobiological responses are the result of photochemical and /or photophysical changes after the absorption of non-ionizing electromagnetic radiation [58].

Figure 1 The mitochondrial respiratory chain. (Figure adapted from [76]).

Production of nitric oxide (NO) in mitochondria especially in injured or hypoxic cells can inhibit respiration by binding to cytochrome c oxidase and displace oxygen [84]. This binding is proposed to dissociate by the PBM or LLLT effect, and reverse the mitochondrial inhibition of respiration due to excessive NO binding [85]. The photobiomodulation effect of LLLT is able to occur a shift in the overall cell redox potential in the direction of greater oxidation by generating reactive oxygen species (ROS) and inhibiting reactive nitrogen

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species (RNS) [86-90]. The excited mitochondrial cytochrome c oxidase after absorbing NIR radiation photon generates ROS that causes changing the oxidation state of the mitochondrial membrane [91].

For the phototherapeutic effect to be observed, the appropriate wavelength of light and dose (fluency) of radiation are needed [83]. However, phototherapy will not be effective on every system and in every situation. Karu (1989) [2] has emphasised that the magnitude of the phototherapy effect depends on the physiological state of the cell at the moment of irradiation [2].

5. Light Emitting Diodes (LEDs)

A light emitting diode (LED) is a semiconductor light source [92]. Henry J Round was the first who reported of light emission from carborundum (raw silicon carbide) in 1907. Oleg Losev, as a lot of people today believe, was the actual inventor of LED. He published his first paper in 1927 on emission of silicon carbide diodes. Losev set up the current threshold for the onset of light emission from the contact point between a silicon carbide crystal and a metal wire and recorded the spectrum of this light [81, 93]. A LED is formed by p-n junctions (p-positive, n-negative), but not all semiconductors are suitable for use as LEDs [94]. The physical mechanism by which LED emits light is spontaneous emission [94]. They emit near-monochromatic, incoherent light [95], in a process called electroluminescence [96]. LEDs are small, robust devices that emit a narrow band of electromagnetic radiation from the ultraviolet to the visible and infrared parts of the spectrum, from around 240 nm up to around 950 nm, according to their electronic structure [95], with a linewidth of around 10-30 nm. LEDs have been publicised as a comfortable, potentially highly selective light-based therapies for many indications [97]. LEDs are also very controllable as light sources for non-thermal applications, acquiring a broad area of in medical applications [61].

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7 Not all light is the same or has equal medical benefits (LED or LASER therapy). Recently,
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9 controversy has arisen around the comparison between low level laser therapy and light
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11 emitting diodes, which have completely different biological effects [98]. A number of studies
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13 compared the effectiveness of LLLT to LED light (Figure 2), and the majority found,
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15 although lasers have small focused spots so only a small area of tissue (< 1 cm^2) is exposed to
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17 light; on the hand LEDs usually have a large area (100 cm^2) so much more tissue is exposed
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19 to light however, lasers are far more effective [98]. Laser therapy can achieve much greater
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21 and deeper stimulative and therapeutically beneficial effects. Laser beams are easily
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23 manipulated using Gaussian beam optics, a simple analytical tool, to enable a laser beam to
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25 be fully controlled spatially, position, size etc. While an LED is difficult to control in terms
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27 of position and spot size, and so it is limited for treatment of superficial tissue only. However,
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29 LED light has some beneficial effect where it is believed that LED light can have a photo-
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31 modulation effect on certain cellular and sub-cellular receptors. In addition, they have greater
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33 choice of wavelengths, are low cost and suitable for acute and chronic conditions [99].
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37 **Figure 2.** Coherent sources and non-coherent (LED) of LLLT in clinical and laboratory
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39 studies on the effect of LLLT on cell and DNA from 1965-2018
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43 A number of studies have been published comparing these two modalities. Kubota and
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45 Ohshiro [100] treated rat skin flaps with an 830 nm GaAlAs laser and an 840 nm infrared
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47 LED. They found an increasing flap survival area in a rat model after being irradiated with
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49 830 nm laser. Flaps treated with the laser had better perfusion, a greater number of larger
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51 blood vessels, and significantly enhanced flow rates. While, flaps treated with an 840 nm IR
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53 LED showed no difference from the control group [100]. Berki et al. [101] used a HeNe
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55 laser to stimulate cell activation in vitro. They observed increasing phagocytic activity along
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with immunoglobulin secretion, but this effect was not seen after irradiation of the cell cultures with LED light of the same wavelength and doses [101].

A comparative study has been performed by Haina et al. [102] to show the effectiveness of HeNe, coherent laser compared with incoherent light of the same wavelength. Experimental wounds were 'punched out' in the muscle fascia of 249 Wister rats. They reported increasing granulation of tissue in the HeNe treated group, whereas there was less granulation in the incoherent light therapy group [102].

Rockhind and colleagues [103] conducted a study comparing five different wavelengths lasers. They gave a single transcutaneous irradiation dose to injured peripheral nerves. They observed reduced subsidence in functional activity following crush injury after HeNe laser irradiation. While the 830 nm IR laser was less effective, the 660 nm incoherent light was even less effective; 880 nm and 950 nm incoherent lights were completely ineffective [103]. Laasko et al. [104] treated patients with chronic pain using an 820 nm IR laser at 25 mW, a 670 nm laser at 10mW and a 660 nm LED. They found an elevated level of ACTH and beta endorphin in the laser therapy groups but not in the LED group [104].

The effect of HeNe laser and incoherent LED light on leukocytes in migration inhibition assays has been studied by Lederer et al. [105]. They reported that irradiation with HeNe laser light affected leukocytes. While, incoherent light of the same wavelength and power density showed no influence [105]. Al. et al. [106] investigated the role of coherent laser therapy in wound healing. They noticed that HeNe lasers with a dose of $1 J/cm^2$ produced an acceleration of the healing process, but incoherent light of the same wavelength and dose was less favourable [106].

Other studies have indicated many reasons which could lead to a preponderance of LED light than to laser light. NASA has stepped into developing LED light therapies for accelerating wound healing, photodynamic cancer treatment and much more. According to NASA: "The

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near-infrared light emitted by these LEDs seems to be perfect for increasing energy inside cells. This means whether you're on Earth, in a hospital, working in a submarine under the sea or on your way to Mars inside a spaceship, the LEDs boost energy to the cells and accelerate healing"[99, 107]. Oliveira and colleagues [108] studied the effect of low level light therapy on the healing of cutaneous wound and their impact on fibroblastic activity during wound healing. They showed an increasing number of healthy animals after irradiation with laser light, and a higher increase was seen when irradiated with LED. They concluded that using LED light caused a considerable bio-modulation of fibroblastic proliferation on anaemic animals. While laser light was more effective on increasing proliferation on non-anaemics [108]. A clinical study by Esper and colleagues [109] was carried out to show the effect of two phototherapy protocols on pain control in orthodontic procedure. They found that LED light therapy had a significant effect in the reduction of pain levels compared to laser light therapy. LED therapy showed a significant reduction in pain sensitivity (an average of 56%), when compared to the control group [109].

Dall et al. [110] performed a comparative analysis of coherent laser light versus incoherent (light emitting diode) light for tissue repair in diabetic rats. They found that the coherent and incoherent lights produced similar effects during a period of 168 h after the lesions had been made. For the control group composed of diabetic animals, 72 h after creation of the lesion, it was observed that the therapy with LEDs had been more efficient compared with the laser for the reduction of the healing period [110]. Similar findings have been obtained by Klebanove and colleagues [111] in a comparative study of the effect of laser and light emitting diode irradiation on healing and functional activity of wound exudate leukocytes [111]. They deduced that coherent laser and incoherent light-emitting diode radiation have very similar effects on wound healing and activity of wound exudate leukocytes, and that the coherence of light is not required for this activity [111]. Another study by Klebanove and colleagues [112]

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has been carried out to explore the comparative effects of laser light and light emitting diodes on the production of superoxide dismutase and nitric oxide in wound fluid of rats. The study indicated that dose-dependent changes in superoxide dismutase activity and production of nitrites in wound fluid after irradiation with visible coherent laser and incoherent LED and the radiation coherence does not play any significant role in the changes of superoxide dismutase activity or nitrogen oxide formation [112].

The rapid evolution of light emitting diodes makes feasible the use of LEDs for medical treatment and light therapy [113]. The single frequency laser does not diffuse, whereas the LED light does. This diffusion allows the cell to be in control of the treatment [114]. Moreover, LED light therapy has been considered non-significant risk by the FDA [81]. For this reason it was published that using light emitting diodes for treatment is much safer than laser therapy [114].

Given the above information, and from recently published studies [115, 116], it has been shown that lasers have an important role in many medical conditions with many positive research results [90, 117, 118], as well as LEDs which are also important in many cases of disease [119, 120]. Nevertheless, in most comparative studies that used laser and LED with the same qualities (wavelength, doses, intensity), it is confirmed that LASERs offer many advantages compared to LEDs [121].

6. Effects of LLLT at Cellular Level

To assess the influence of low level laser therapy at the cellular level, cell cultures are one of the best biological systems used to find out the effect of laser irradiation on cell proliferation rate. Various studies, which have used different types of laser therapy with a variety of cells, have been designed to improve understanding on the effect of LLLT at the cellular level

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(Figure 3). More recent studies have studied the bio-stimulatory effect of low level laser on cell proliferation processes.

Early work by Karu and colleagues [122] have reported that the cytotoxic response of Hela cells to ionizing radiation can be influenced by irradiation with He-Ne laser 632.8 nm with an energy density 100J/m^2. They observed that there was a substantial difference between the survival curve of Hela cells treated with He-Ne laser for 60 min before exposure to γ-irradiation and the curve representing the survival of untreated γ-irradiated cells. Moreover, an increase in the number of cells has been observed after stimulation with a He-Ne laser compared to the control group [122].

Pereira and colleagues [123] examined a 632.8 nm He-Ne laser with an energy fluence of 0.053 to 1.89 J/cm^2 and a 904 nm (GaAs) laser with an energy fluence of 1.94×10^{-7} to 5.84×10^{-6} J/cm^2 on fibroblast cell cultures, which determined by using the Trypan blue dye exclusion assay. No difference in cellular proliferation for fibroblast cells exposed to a He-Ne laser versus untreated fibroblast cells could be found. On the other hand, with GaAs laser, a decrease in cellular proliferation of fibroblast cells compared to controls was observed. However, both He-Ne and GaAs lasers induced procollagen production [123].

It was noted that with exposure to a 670 nm GaAlAs laser, an increase in myofibroblasts and collagen deposition was observed [124]. Furthermore, an increase in gingival fibroblasts after exposure to diode lasers (670, 692, 780, and 786 nm) was also found [4].

Bouma and colleagues [125] examined human monocytes and human umbilical vein endothelial cells (HUVECs) with a 904 nm GaAs laser at 40.18 mW/cm^2 power density. They found no difference in the cytokines level such as tumour necrosis factor TNFα, interlukin-6 and -8, E-selectin, intercellular adhesion molecule 1, and vascular cellular adhesion molecule 1 [125]. Schindl and colleagues [126] reported that HUVECs irradiated

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with a 670 nm diode laser with a dose of 2 to 8 J/cm^2 resulted an increase in the proliferation of these cells, that determined by using a haemocytometer [126]. An in vitro study by Hass and colleagues [127] showed an increase in human keratinocytes mortality, that observed by inverted phase microscopy after exposure to He-Ne laser and found no change in proliferation or differentiation [127]. While, Grossman and colleagues [128] observed an increase in proliferation rate of keratinocyte cells, which counted microscopically using a counting chamber after exposure to a 780 nm continuous-wave diode laser with a dose from 0 to 3.6 J/cm^2 [128].

Figure 3. Light sources used in clinical and laboratory studies on the effect of LLLT on cell functions from 1965-2018.

Researchers pointed out that using low laser therapy with low doses can increase the proliferation rate of cultured cells when compared to high doses. Beyond a certain dose level, which is cell type dependent, high dose levels have a detrimental effect on cell proliferation rates. AlGhamdi and colleagues [59] have examined stem cells with a He-Ne laser at 632.8 nm and a GaAlAs at 600 nm, with a range of energy densities (doses) from 0.5 – 4.0 J/cm^2 and power densities from 1-500 mW and found that LLLT can increase the proliferation rate of various cell lines. They have confirmed that the stimulation of cellular proliferation is dependent on the dose level of laser irradiation. They concluded that lower doses increase the rate of cell proliferation and other cellular functions, the determination of cell count and was achieved by using Trypan blue stain. Whereas, higher doses of low level laser therapy have negative effects, where the high doses caused a significant decrease in cells count and the percentage of cell viability [59]. Similar results have been obtained by Walsh and colleagues [129], when they irradiated fibroblasts of skin cells, buccal mucosa and gingival cells with semiconductor lasers at 540 nm and 600-900 nm and energy densities 0-56 J/cm^2. Walsh noted increased cell proliferation at low doses, which measured by using Trypan blue dye

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exclusion assay, and repressed at high doses. They, also observed increase maturation and locomotion, transformation to myo-fibroblasts, and increased production of basic fibroblasts growth factors

Walsh and colleagues [129] used the same laser with the same energy densities to examine macrophage cells. They observed convergent results, greater secretion of basic fibroblasts growth factors, increased ability to act as phagocytes, and resorption of fibrin by macrophages. Walsh in another study used semiconductor lasers of 660, 820, and 940 nm to treat human lymphocytes cells. They showed activated lymphocytes and high proliferation rate. With the same wavelengths, Walsh noted the increased motility of epithelial cells and an ability to migrate across wound sites with quickened closure of defects.

Unlike AlGhamdi and Walsh, Petri and colleagues [130] found that cell growth, as measured by MTT assay, was affected by time with LLLT after exposing human alveolar bone fragment cells to a GaAlAs diode laser of 780 nm with power of 70 mW and energy density 3 J/cm^2 [130]. Recently, Forouzanfar [131]has support Petri's results when examining human gingival fibroblasts with a Ga-Al-As diode laser at 810 nm, output power 50 mW and energy density 4 J/cm^2 . Forouzanfar noted that both good levels of cell proliferation and secretion of macromolecules can be regulated if enough exposure time of low level laser therapy has been given to the cells to determine whether LLLT could induce a bio-stimulatory effect on human cells. As well, they have found a significant difference between the case and control groups on 48 and 72 hr after irradiation [131].

Tuby and colleagues [132] obtained a positive result when they exposed mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs) to a GaAs diode laser at 804 nm with an energy density between 1 and 3 J/cm^2 and an output power 50 mW. The results showed a significant increase of seven-fold and two-fold in the number of CSCs after 1 and 2 weeks post

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irradiation of 1 J/cm^2 for 20 sec exposure and increased the number of MSCs and CSCs after 1 week post irradiation of 3 J/cm^2 compared to the control [132].

Almeida and colleagues [133]used diode laser with 670, 692, 780, and 786 nm wavelengths and fluence (energy density) of 2 J/cm^2 to show the comparison of LLLT effects on the proliferation rate of cultured human gingival fibroblast cells. They found that in the same fluence and with different output powers, infrared lasers induced a higher proliferation rate of cells compared to visible laser. Whilst lasers of equal output power were shown to have similar effect on cell growth independently of their wavelengths[133].

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7. Effect of LLLT at Molecular Level

LLLT has been in existence for more than four decades. It has been found beneficial in a wide variety of therapeutic applications [57]. However, the possibility of induced DNA damage has now arisen; even though, this damage could be repairable [57]. Although, phototherapy is used in the biomedical treatment of many diseases, the mechanisms of laser-molecule interaction remain unclear and the deleterious effects of laser irradiation are still controversial [134].

LLLT is usually performed with visible red or near infrared laser light and with typical accumulated doses. Since employing wavelengths within the red side of the optical spectrum, which is likely to be less damaging to DNA than sun light, it is assumed that the doses per area of LLLT are safe when corresponding to the DNA damaging effects of a few minutes sunlight [134]. If such irradiation induces DNA breaks, these breaks are likely to be repaired immediately; otherwise unrepaired damage could lead to mutations consequently leading to development of cancer in the long run [135].

Different studies in eukaryotic and prokaryotic cells have reported adverse effects on cells and DNA damage after exposure to low power laser therapy [136] (Figure 4). Experimental

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data about the effect of these light sources with different power, wavelengths, and emission modes on DNA are however scared [78]. A study by Zhang and colleagues [137] using microarray technologies indicated that low intensity laser exposure (red light) at therapeutic doses has been demonstrated to promote expression of DNA repair genes following DNA lesions induced by free radicals [137].

Figure 4 Light sources for LLLT used in clinical and laboratory studies on the effect of LLLT on DNA from 1980-2018.

It has been reported that the photo-reactivating enzyme (DNA photolyase) distinguishes one type of DNA damage as its substrate (i.e. the cyclobutane-type pyrimidine dimer), and combines with these dimers in the dark [58]. However, when exposing the enzyme-substrate complex to visible light, the enzyme uses the absorbed energy of light to split the dimer to produce repaired DNA. Mbene [57] treated wounded human skin fibroblast cells by He-Ne laser with 5 J/cm^2 and 16 J/cm^2 doses. Irradiation with 5 J/cm^2 and 16 J/cm^2 showed insignificant change in DNA damage, as determined by alkaline comet assay, at 1h when compared to their respective controls. However, a significant decrease in DNA damage at 24h incubation due to the mechanism of DNA damage repair was shown [57].

Fonseca and colleagues [22] irradiated E.coli cells with low intensity (AlGaInP) red laser with a power of 10 mW and with different fluencies (1, 4 and 8 J/cm^2). It was suggested that low-level red laser light induces DNA lesions as a result of the generation of free radicals. They suggested that biological effects induced by low level laser fluence could occur due to the generation of free radicals. They suggested that considerable importance should be given to low-level lasers for their potential to induce DNA repair and changes in gene expression profile of the irradiated cells [22].

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A study by da Silva and colleagues [138]used an AlGaInP laser with a power output of 10 mW, and with continuous or pulsed mode of irradiation. They found that low-intensity red laser radiation could induce DNA lesions via oxidative mechanisms. Moreover it was found that the survival mechanism against harmful radiation could be activated or induced after irradiation with monochromatic red light [138]. Kohli and colleagues [139] examined E.coli cells with a He-Ne laser at 632.8 nm. They observed that irradiation with low level He-Ne lasers induces photolyase gene (*phr*) and DNA repair genes investigated by *phr* gene expression assay. The magnitude of induction relies on fluence rate of the He-Ne laser and the time of incubation post irradiation. The study concluded that the stimulation of DNA repair may explain the higher survival cell against UV radiation [139].

Dube and colleagues [140] studied the effect of He-Ne laser 632.8 nm pre-irradiation on UVA induced DNA damage in the human B-lymphoblast cell line, as measured by comet assay. They found a decrease in UVA-induced DNA damage. Whereas, the control cells showed higher DNA damage, the same rate of DNA damage in He-Ne laser pre-irradiated cells. The results suggest that He-Ne laser irradiation plays an important role in protecting the cells from UVA-induced DNA damage primarily through an influence on processes of preventing an initial damage of DNA [140].

Dillenburg and colleagues [141] triggered epithelial cells with laser phototherapy (LPT) of energy density 4 J/cm^2 and 20 J/cm^2. They observed that laser phototherapy at a low energy density of 4 J/cm^2 did not induce DNA damage or genomic instability, that determined by comet assay. Interestingly, a low energy of LPT induced nuclear influx of the BRCA1 protein of DNA repair, which is a genome protective molecule that effectively takes part in DNA repair. Importantly, these findings suggest that LPT of low dose induces a safe level of reactive oxygen species (ROS), which accelerate healing [141].

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Ridha and colleagues [142] used a He-Ne laser 632.8 nm to irradiate human lymphocytes. They concluded that the effect of low red laser light in maintaining cell survival may be attributed to the induction of endogenous radioprotectore and improvement of DNA repair due to induce enzymes involved in repair process [142]. More recently, Trajano and colleagues [143] stated that at therapeutic fluences, exposure to red visible laser therapy alters the expression of genes related to the base excision and nucleotide excision pathways of DNA repair during wound healing [143].

Although, most of the aforementioned studies have been appeared to show the effect of LLLT on cell proliferation, conflicting results have been published. As well, studies tried to explain the induction effect of LLLT on repair mechanisms of DNA damage showed variance results. All these contrasts may be related to a disparity in study design, including the use of different lasers, variations in parameters such as energy densities, wavelengths, exposure time, output power etc.

8. Discussion

Interest in the field of LLLT has been rekindled in concert with philosophical evolution toward minimally invasive laser therapies [144]. Although the action of lasers on biological tissue is mediated via photothermal effect, LLLT ideally causes low or imperceptible temperature changes, making LLLT known as" low intensity" or" cold" lasers [41]. Experiments of measuring the temperature following LLLT exposure have shown that the immediate increase in temperature of the irradiated tissue is negligible (±1℃) [145]. Many researchers emphasises that the temperature remained unchanged in suspensions of different cells through LLLT irradiation [146, 147]. Studies by Schneede and colleagues [148] suggested that the temperature could raise by less than 0.065℃, during irradiation with laser of 40 mW/cm^2, they used a microthermal probe in a monolayer of cells to measure the temperature [148].

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Lasers are distinctive and their unique properties of diffraction limited spot of sub-micro dimensions, yielding high power density, ultrashort pulses, coherent radiation (i.e., the light waves are all in phase), and monochromaticity are all made use of [77]. However, many researchers have been found no significant difference for photo stimulation regardless of whether the light used was generated by a laser source or from light of the same wavelength from a filtered incandescent lamp. This review shows an increasing number of papers in the literature on photo therapy in recent years using incoherent light sources, such as LEDs [77, 149].

These findings build on previous reviews of LLLT by including biological effects of LLLT at cellular and molecular levels. Although, various studies included hypotheses explaining the mechanisms of laser action on biological systems, the understanding of the biological effects of laser therapy is still poor. This review has identified a growth in the number of studies. Many studies, often with conflicting results in this field have been published [58]. These discrepancies may be attributed to a variance in study design, including the use of different lasers and inequalities in parameter selection. As well as this, it may, as Karu (1989) indicated, relate to the physiological state of the cell at the moment of irradiation [58, 150]. In general, for laser studies to be useful, all the characteristics of the light emitted from laser source or by LEDs must be specified [77].

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9. Conclusions

In conclusion, LLLT is a treatment method using laser light of low energy or intensity. It delivers a very low energy, enough to produce stimulation, but not destruction of the target system; therefore, it has been used extensively for diverse studies. Applications of this optical tool have also attracted criticism with respect to its reproducibility despite, several advantages. The present review has highlighted many subjects included the emergence of

LLLT, the mechanism of LLLT interaction with the biological system, the optical properties of tissue, the cellular and molecular effect of LLLT as well as, the types of lasers used for LLLT. However, it emerges that most studies concern dose and wavelength. There have only been a limited number of studies so far on the physical parameters of LLLT such as coherence and polarisation of light. The outcomes of this review revealed that, in addition to low intensity coherent lasers, incoherent light emitted from LED, is used widely, with a wide range of therapeutic applications. There were conflicting views as to whether coherent laser or incoherent LED has the most beneficial therapeutic impacts on biological systems. In spite of the large number of studies including different laser types, studies using the same parameters of LLLT to assess cell survival or effects on DNA are so far almost non-existent More studies using LLLT with different properties are needed to investigate which laser with specific properties has a beneficial effect on biological system, in order to be included within the therapeutic tools, and which has a deleterious impact to be excluded from uses (e.g., to treat malignant problems). Furthermore, local magnetic field as magneto-optical phenomenon can change the polarisation dependent absorption of laser light. These aspects need further studies in relation to therapeutic uses of LLLT.

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Conflict of interests

Authors have no conflicts of interest.

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Table1. Review of published studies using Low Level Laser Therapy (LLLT) to treat different diseases.

Study No.	Type of laser	Wavelength (nm)	Power (mW)	Energy density (J/cm^2)	Power density (mW/cm^2)	Emission model CW / Pulse	Types of diseases	Reference
1	Diode laser	810	10 W	3 and 30	5 and 50	CW	Zymosan-induced arthritis	Castano et al. [16]
2	He – Ne	632.8	10	3, 5, 10, 20, 25 and 50	64.6	CW	Neurodegenerative	Song et al. [17]
3	He – Ne	632.8	10	0.5, 1, 2 and 4		CW	Alzheimer's disease	Meng et al. [18]
4	Nd:YAG	1064	1.25 W			CW	Dental/Tooth extraction	Vescovi et al. [19]
5	GaAs	904	10	5.4	20	CW	Musculoskeletal diseases	Bjordal et al. [20]
6	Diode laser	830	30	1.1		Pulse	Painful stomatitis control	Toida et al. [21]
7	Diode laser	810	30	0.9	30	CW	Diabetic wounds	Dancakova et al. [22]
8	Diode laser He – Ne	830 632.8	30 20			CW	Chronic diseases of inner ear	Wilden et al. [23]
9	Diode laser	660	50	2		CW	Chronic lichenoid graft-vs.-host disease (cGVHD)	Chor et al. [24]
10	Diode laser	810		3	20	CW	Cortical neurons	Huang et al. [25]
11	He – Ne	632.8	400	1		CW	Alzheimer's Disease	Farfara et al. [26]
12	GaAlAs	860	30 60	3	3000	Pulse CW	Osteoarthritic (OA) pain	Brosseau et al. [27]
13	GaAs	808			10 and 20	CW	Traumatic brain injury (TBI)	Oron et al. [28]
14	GaAlAs	830	60	45	4000	CW	Lumbago	Ohshiro et al. [29]
15	Diode laser	660	30	7.5		CW	Lung neutrophils	Aimbire et al. [30]
16	Diode laser	660	40	20		CW	Burning mouth syndrome	Santos et al. [31]
17	Diode laser	665, 730 810 and 980		36	150	CW	Traumatic brain injury (TBI)	Wu Qiuhe et al. [32]
18	Diode laser	660	24			CW	Periodontal disease	de Almeida et al. [33]

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Table1. Continued

19	**Diode laser**	**820**	**300**	**3**		**CW**	**myofascial pain (MP) dysfunction syndrome**	**Oz Selcen et al. [34]**
20	GaAlAs	780	50	7.5		CW	Rheumatoid arthritis	Ekim et al. [35]
21	Diode laser	810		0.03, 0.3, 3, 10 and 30	25	CW	Cortical neurons	Sharma et al. [36]
22	GaAlAs	830	70	6		CW	Peripheral nerves regeniration	Midamba et al. [37]
23	GaAlAs	810	1 W	4.8 24	80	CW	Orofacial granulomatosis	Merigo et al. [38]
24	Diode laser	830	100	3		CW	Chronic periodontitis	Makhlouf et al. [39]
25	Diode laser	780 830	30 500	6.3 100		CW	Temporomandibular joint pain	Chang et al. [40]
26	He - Ne	632.8	10	0.18 - 27		CW	Indolent ulcers	Schindl et al. [41]
27	Diode laser	808			110 165	CW	Hearing loss	Tamura et al. [42]
28	Diode laser	532 635	7.5			CW Pulse	Hearing loss	Goodman et al. [43]
29	Diode laser	650	5			CW	Complaints of Tinnitus	Salahaldin et al. [44]
30	InGaAIP	660	10	2.5		CW	Acute zymosan-induced arthritis	Carlos et al. [45]
31	GaAs	904	20	2 - 20	11.2	Pulse	chronic myofascial pain syndrome (MPS) in the neck	Gur et al. [46]
32	GaAs	904		29.5	246	Pulse	Salivary Glands (Xerostomia)	Loncar et al. [47]
33	Diode laser	630 – 670 780 – 830	10—100	2, 3 and 4		CW	Oral mucositis due to cancer therapy	Bensadoun et al. [48]
34	Diode laser	660, 810 and 980		36		CW	Traumatic brain injury (TBI)	Wu Qiuhe et al. [49]
35	GaAlAs	670	5	2		CW	Chronic periodontitis Diabetes mellitus (DM)	Obradovic et al. [50]
36	Ga-Asl-Al	780	22	7.7	100	CW	Rheumatoid arthritis (RA)	Alves et al. [51]

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Table1. Continued

37	**Diode laser**	**810**		**36**	**50**	**CW**	**Traumatic brain injury (TBI)**	**Xuan et al. [52]**
38	Diode laser LED	685 640 – 685	200	2		CW	Reynaud's phenomenon	Hirschl et al. [53]
39	Diode laser	810			50	CW	Parkinson's disease (PD)	Trimmer et al. [54]
40	Diode laser	790	120	6		CW	Burning mouth syndrome	Kato et al. [55]
41	IR laser	830	35	3		CW	Lung inflammation	Oliveira et al. [56]
42	GaAs	904	150	6		Pulse	Carpal tunnel syndrome	Dakowicz et al. [57]
43	AlGaAs	780	30	22.5	750	CW Pulse Pulse	Renal Interstitial Fibrosis	Oliveira et al. [58]
44	GaAlAs	830	60	18	3000	CW	Knee Osteoarthrosis	Trelles et al. [59]
45	AlGaAs	785	70	3		CW	Rheumatoid arthritis	Meireles et al. [60]
46	Diode laser	670	50	3		Pulse	Temporomandibular disorder (TMD)	Nunez et al. [61]
47	GaAs	904	45	5		CW	Muscle trauma	Rizzi et al . [62]
48	GaAlAs	980	300	4	1500	CW	Mucous membrane pemphigoid	Cafaro et al. [63]
49	Diode laser	660	5	4.5		CW	Acut Lung inflammation	de Lima et al. [64]
50	GaAs	980	10 80 W	2-4		CW Pulse	Chronic low back pain (LBP)	Hadi et al. [65]
51	GaAlAs	980	300	4	1000	CW	Oral lichen planus	Cafaro et al. [66]

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Table1. Continued

52	**GaAlAs**	**660**	**30**	**57.14**	**428**	**CW**	**Periodontal disease (PD)**	**Garcia et al. [67]**
53	InGaAlP	660	40	2	1000	CW	Ulcers in patients with leprosy sequelae	Barreto et al. [68]
54	GaAlAs	815	250	12		CW	inflammation in retrodiscal tissues in patients with temporo mandibular joint	Kucuk et al. [69]
55	GaAlAs	808	500	5	1.8	CW	Bisphosphonate Related Osteonecrosis of Jaws	Altay et al. [70]
56	AsGaInP	660	50 100	12.5 25	1.25 2.5	CW	Third-Degree Burns	Brassolatti et al. [71]

Table 2: Parameters involved in Low Level Laser Therapy (LLLT).

Irradiation Parameters	Unit of measurement		Definitions
Wavelength	nm	390– 10,600	An electromagnetic radiation travels in discrete packets that also have a wave-like property.
Power	W	$10^{-3} – 10^{-1}$	It is the amount of energy consumed per unit time, and can be calculated as: Power (P) = Energy (J) /Time (sec)
Power density	W / cm^2	$10^{-1} – 10^{0}$	Often called Irradiance, or Intensity, is the power transmitted per unit area, and calculated as: Power density = Power (W) / Area (cm^2)
Energy density	J / cm^2	$10^{-2} – 10^{2}$	Energy density is the common expression of LLLT dose The dose is the most important parameter in laser Phototherapy, and is usually calculated as Power / Beam Area x Time = J/cm².
Total irradiation time	sec	10 – 3,000	Is the allowed interval through which the energy has delivered to the target system.

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Table 3. Review of published studies evaluating the effect of LLLT on different cell lines.

No	Cell Types Used	How the cells are grown	Type of LLLT	Quality of Laser Used	Biological Effects Determination	References
1.	Human skin fibroblast cells	Cultures in minimum essential medium with Earl's balanced salt solution & incubated in 37℃ in 5% & 85% humidity	He-Ne Laser	λ: 632.8 nm Energy density (ED) 5J/cm^2	1) Non irradiated Hydroxyuria (HU) treated cells had a reduced number of cells in the central scratch compared to non-irradiated non treated cells, suggesting that HU inhibited cellular proliferation. 2) Irradiated HU treated cells showed an increased number of cells in the central scratch compared to non-irradiated treated cells. This increase was due to the stimulatory effect of irradiation with 5 J/cm2. The addition of HU had no significant effect on cell viability. 3) The Trypan blue exclusion test showed no significant difference in percent viability between treated and non-treated cells. 4) Irradiated non treated cells showed a significant increase in the formazan dye, which is as a result of cleavage of XTT by the mitochondrial succinate dehydrogenase in actively proliferating cells, compared to non-irradiated non treated cells. 5) Cell viability, proliferation and DNA integrity assays showed that irradiated and non-irradiated N cells were not significantly affected at both 1 and 24 h post irradiation. 6) there was a significant decrease in damage at 24 h compared to 1 h incubation due to the activation of DNA repair mechanisms.	Mbene et al. [108]
2.	E.Coli AB1157, BW527, BW9091 and BW375	Cultures in exponential and stationary growth phase. *E. coli* suspensions (1-2 × 10^8 cells/mL, in 0.9% NaCl solution)	Laser HTM Compact model, AlGaInP	Power:10 mW λ: 658 nm	1) There is no alteration of survival fractions of these E. coli cultures when exposed to laser. 2) I was indicate that laser exposure induces filamentation in exponential E. coli AB1157, BW527, BH20, BW375 and BW9091 cultures at all emission modes	da Silva et al. [109]

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Table 3. Continued

					3) Laser – induced stimulation of cell replication in E.coli cultures depends on the culture conditions, determining the particular metabolic state necessary for the division.	
3.	Stem cells	Does not maintion the culture procedure	He-Ne Laser Gallium-Aluminum-Arsenide (Ga-Al-AS)	λ: 632.8 nm λ:600 nm Energy density: 0.5 - 4.0 J/cm^2 Power 1- 500 mW	1) LLLT can increase enhance the proliferation rate of various cell lines. 2) The stimulation of cellular proliferation is dependent on the doses of laser irradiation, as lower doses increase the cell proliferation rate and other cellular functions, while higher doses of LLLT have negative effects.	AlGhamdi et al. [110]
4.	Mesenchymal stem cells (MSCs) & Cardiac stem cells (CSCs)	Cell cultured at 1.3 × 10^6 cm^2 in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 m mol/L Glutamine, 100 U/ml pencillin, 100 U/ml stroptomycin CSC cultured in a class 2 flow hood.	Diod (Ga-As)	λ 804 nm Power density: 50 mW/cm^2 Energy density: 1 & 3 J/cm^2 Exposure time: 20 sec or 60 sec	1) CSCs of (1J/cm^2) 1 and 2 weeks post LLLT irradiation significant increase of sevenfold and twofold respectively in the number cells compared to control. 2) Significant increase in the number of cells at the energy density 3 J/cm2 after 1 week. 3) The number of MSC$_S$ increased post LLLT of 50 mW/cm^2 for 20 sec and 60 sec	Tuby et al. [111]
5.	Fibroblast of skin cells, buccal mucosa			λ: 540 nm 600 – 900 nm	1) Increased proliferation, maturation and locomotion as well as transformation to myo-fibroblasts. 2) Reduced production of pro-inflammatory prostagland in E2	Walsh et al. [112]

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	and gingival			Energy density: 0-56 J/cm^2	3) Increased production of basic fibroblasts growth factors. 4) Increased proliferation at low doses and suppressed at high doses.	
	Macrophages				1) Increased ability to act as phagocytes, and greater secretion of basic fibroblasts growth factors. 2) Macrophages resorb fibrin as part of the demolition phase of wound healing more quickly with LLLT, because of their enhanced phagocytic activity during the initial phases of the repair response.	
	Lymphocytes			λ: 660 nm 820 940 nm	Lymphocytes become activated and proliferate more quickly	
	Epithelial cells				These cells become more motile and are able to migrate across wound sites with accelerated closure of defects.	
	Endothelium cells				Endothelium forms granulation tissue more quickly. Relaxation of vascular smooth muscles	
6.	Human Gingival Fibroblasts (Hgf3-Pi 53 NCBI code C50)	The cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS). This medium was also supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100µg/ml streptomycin.	(Ga–Al–As) diode laser	λ: 810 nm Power: 50 mW Energy density: 4J/cm^2 Exposure time: 32 sec	1) The differences between the case and the control groups were statistically significant on 48 hr and 72 hr after irradiation. 2) The results of this in vitro study revealed that good levels of cell proliferation could be achieved if enough time has been given to the cells to show the effect of laser irradiation on cell proliferation rate.	Frozanfar et al. [113]

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7.	HeLa cells	They were grown as monolayers in scintillation vials	He-Ne laser	λ: 632.8 nm Power density: 10 W/m^2 Exposure time: 10 sec Energy density: 100 J/m^2	1) When the cells exposed to laser radiation for 60 min before exposure to γ-radiation, substantial differences was seen between the survival curve and the curve representing the survival of γ-irradiated cells. 2) Increased the number of cells after stimulation with He-Ne in the exponential phase of growth than that for the control.	Karu et al. [114]
8.	Yeast, HeLa		He-Ne laser	λ: 632.8 nm Power density: $I \geq 2\times10^{11}$ W/cm^2	The activity of some enzymes was determined and shows that the growth stimulation is accompanied by the respiratory activity increase with no accumulation of toxic intermediates of oxygen metabolism and by synthetic processes in cell predominance over degenerative once. The data indicated that the irradiation causes a cell metabolism rearrangement, the light playing the role of a trigger controller of the cell metabolism.	Karu et al. [115]
9.	Human B-lymphoblasts	Human B-lymphoblast cells (NC 37) were grown in suspension in RPMI 1640 medium (Sigma, Germany) with 10% fetal calf serum at 37°C in a 5% CO2 atmosphere. The cells were sub-cultured twice weekly in fresh RPMI	He-Ne laser	λ: 632.8 nm Power: 10 W Diameter of beam: 0.75cm Doses ranging 0.5-2.7 kJ/m^2	1) The cell viability measurement shows no significant change of the cell survival. 2) He-Ne lasers alone do not result in any DNA damage.	Dube et al. [116]

		1640 medium.				
10.	Human alveolar bone fragments	Cells were cultured in α-Minimum Essential Medium (Gibco), supplemented with 10% fetal bovine serum (Gibco), 50 µg/mL gentamicin (Gibco), 0.3 µg/mL fungizone (Gibco), 10-7 M dexamethasone (Sigma, St.Louis, MO, USA), 5 µg/mL ascorbic acid (Gibco), and 7 mM β-glycerophosphate (Sigma)	GaAlAs diode laser	λ: 780 nm Power: 70 m W Diameter of beam 0.2 cm Energy density: 3 J/cm^2 Exposure time: 9 min	1)Cell growth was affected by time only in LLLT group 2)From day 10 to 14, LLLT treated cultured showed an increase of cell growth	AD Petri et al. [117]
11.	human gingival fibroblasts	A cell line of human gingival fibroblasts named LMF was grown in DMEM with either 5% nutritional deficit or 10% (FBS)	Diode laser	λ: 670 nm, 780nm, 692nm 786nm Energy density (fluence) 2 J/cm^2 Exposure time: 9 min	1)The irradiated cell number of cell cultured in 5%nutrition deficit more than that control cell cultured in idial conditions 2) In the same fluence, IR laser induced a higher cell proliferation than visible laser when the output powers are different. 3) Lasers of equal output power presented the similar effect on cell growth independently of their wavelength.	Almeida et al. [118]
12.	Human Macrophages	The macrophage J774 cell line was grown in (DMEM) supplemented with 10%	Diode laser	λ: 780 nm Power: 70 mW	1) After 1 day of culture, activated and 780 nm irradiated macrophages showed lower mitochondrial activity (MA) than	Souza et al. [119]

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		fetal bovine serum (FBS) and 2 mM L-glutamine at 37°C and in a wet environment with 5% CO2. Cell growth was assessed every 24 hours using an inverted phase microscope		Energy density: 3 J/cm^2 λ: 660 nm Power: 15 mW Energy density: 7.5 J/cm^2	activated macrophages, but activated and 660 nm irradiated macrophages showed MA similar to activated cells. 2) After 3 days, activated and irradiated (660 nm and 780 nm) macrophages showed greater MA than activated macrophages, and after 5 days, the activated and irradiated (660 nm and 780 nm) macrophages showed similar MA to the activated macrophages.	
13.	MG-63	Cells were maintained it in Dulbecco's modified Eagle medium (DMEM) with 100 IU/ml penicillin , 50 µg/ml gentamicin, 2.5 µg/ml amphotericin B , 1% glutamine and 2% HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)) , supplemented with 10% fetal bovine serum. Cultures were kept at 37℃ in a humidified atmosphere of	Diode laser	λ: 940 nm Energy outputs: 1-5 J Intensities: 0.5, 1, 1.5 and 2 W/cm^2	Pulsed low-level laser with low-energy density range appears to exert a biostimulatory effect on bone tissue.	Huertas et al. [120]

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Table 3. Continued

		95% air and 5% CO_2. .				
14.	Osteoblastic (MC3T3) cell line	Cells were grown in sterile Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM = F-12) (Invitrogen, Mount Waverley, Australia) supplemented with heat-inactivated fetal bovine serum (FBS) (Cambrex, East Rutherford, NJ), and 200 ml penicillin + 200 mg =ml streptomycin (Invitrogen)	Diode laser	λ: 830 nm Power: 30mW Energy density: 10 J/cm^2	Reduction in cell proliferation compared to non-irradiated controls.	Renno et al. [121]
15.	Human osteoblast cell line	Cells were maintained in sterile medium (Dulbecco's Modified Eagle's Medium): Nutrient Mixture F-12 (DMEM= F-12) (Invitrogen, Mount Waverley, Australia) supplemented with heat-inactivated fetal bovine serum (FBS) (Cambrex, East Rutherford, NJ), and 200 ml penicillin+ 200 mg =ml streptomycin (Invitrogen)	He-Ne laser 632 nm	λ: 632 nm Power:10mW Energy density: 0.43 J/cm^2	LLLT promotes proliferation and maturation of human osteoblasts *in vitro*, and a significant 31–58% increase in cell survival	Stein et al. [122]

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16.	Human monocytic THP-1 cell line	THP-1cells were grown in 50 ml culture flask, the flask containing 20ml of medium plus cell, at 37℃ with 5% CO2 in a humidified incubator.	Diode laser 850 nm	λ: 850 nm Power: 9.5 mW Energy density: (0.6- 27J/cm^2) power density of 29.6 mW/cm^2	PBM promotes proliferation of human monocyte *in vitro*, and a significantly increased cell survival due to increasing membrane integrity and mitochondrial activity.	Ruwaidah et al. [123]
17.	stem cells from exfoliated deciduous teeth (SHED)	Cells were maintained in Eagle's minimum essential medium alpha modification supplemented with 10% FBS and 1% penicillin and streptomycin solution (penicillin–streptomycin, Gibco, Invitrogen) at 37 °C and 5% CO2 in incubator.	InGaAlP red laser	λ: 660 nm Energy density: (1.2- 6.2 J/cm^2)	Improved cell viability and proliferation of SHED after laser irradiation, except for 1.2 J cm–^{2}.	de Souza[124]

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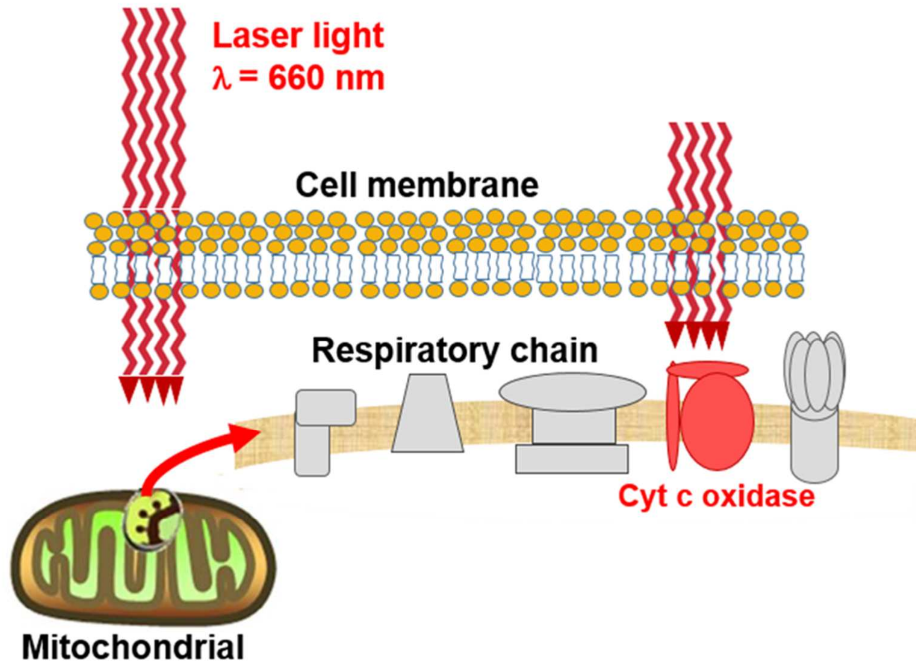

Figure 1. The mitochondrial respiratory chain. (Figure adapted from [148])

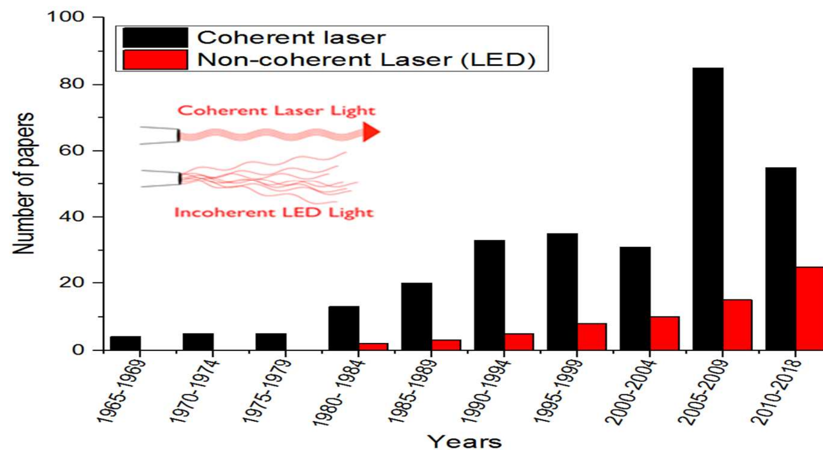

Figure 2. Coherent sources and non-coherent (LED) of LLLT in clinical and laboratory studies on the effect of LLLT on cell and DNA from 1965-2018.

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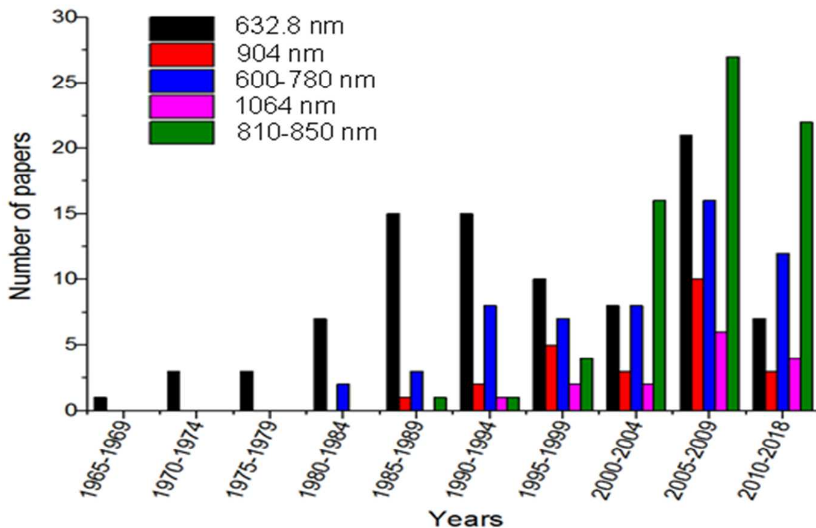

Figure 3. Light sources used in clinical and laboratory studies on the effect of LLLT on cell functions from 1965-2018.

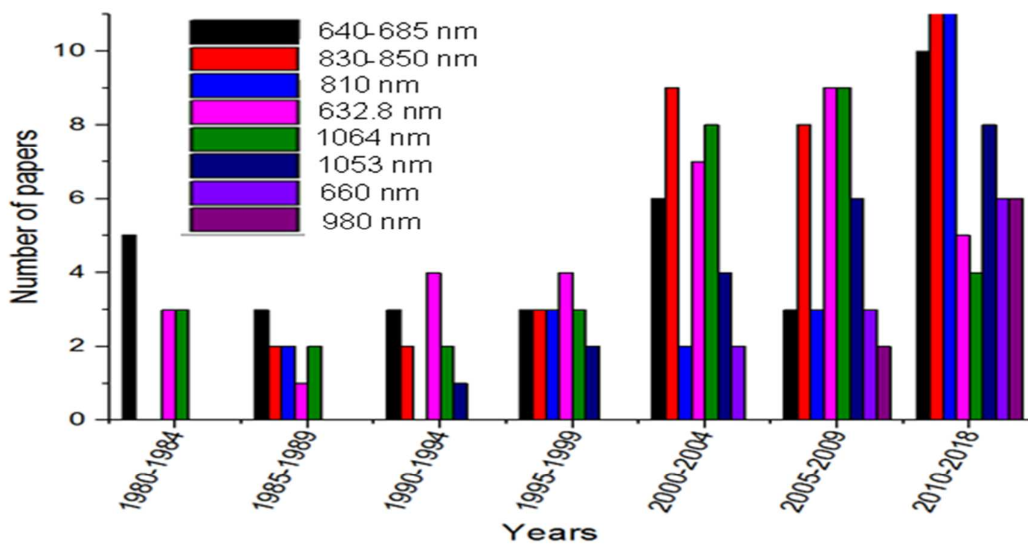

Figure 4. Light sources for LLLT used in clinical and laboratory studies on the effect of LLLT on DNA from 1980-2018.