Assessing the impact of low level laser therapy (LLLT) on biological systems: a review

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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 articles 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 summarizes 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 minimizing its possible detrimental effects.

1. Introduction

Laser therapy or low-level laser therapy (LLLT) has been widely used for over 50 years (Ginani et al. 2015). Evolutionary though, 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 (Karu 1989; Kreisler et al. 2003; Posten et al. 2005). Investigators introduced a diverse set of terms to describe this potentially beneficial treatment tool (Lucas et al. 2002). Initially, expressions such as `photobiostimulation’ and ‘biostimulation’ frequently relative to the stimulation effect of low level lasers were used (King 1989; Wu et al. 2012). Subsequently an inhibitory effect of this radiation were also noted, which led them to coin the term ‘biomodulation’ (Schindl et al. 2000).

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 (Hamblin 2017). On the contrary, other researchers reported that although LLLT is a well-established researchable and for much time used by clinician and researchers, 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 utilize 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 (Anders et al. 2015). Specialists of medical field successfully used photobiomodulation in treating many health conditions when other methods had limited success, such as healing-resistant wound, chronic diabetic ulcers, injuries of spinal cord and nervous system and pain management (Tuner and Hode 2004). Nevertheless, photobiomodulation is not considered as a part of mainstream medicine as still not standard treatment (Karu 2013).

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 (Chung et al. 2012). The therapeutic treatments are based upon three
principles: (a) to minimize inflammation, edema, and chronic disorders of joints by targeting brain, skin, joint, etc. (Bjordal et al. 2003), (b) to promote wound healing of superficial and deeper tissues, neurological damage, etc. (Gigo-Benato et al. 2005; Posten et al. 2005), and (c) to treat neurological disorders and pain (Chung et al., 2012). Recently, many studies on PBM therapy at infrared (IR) wavelengths, in particular from 700 nm up to the near infrared (NIR) have been carried out. (Barrett and Gonzalez-Lima 2013, Xuan et al. 2014, Salehpour and Rasta 2017). These studies have shown to produce more beneficial impacts than red light in many medical conditions. These include neural stimulation (by triggering direct activation of neural tissue) (Salehpour et al. 2017), photoagging (where IR radiation evidently has a biphasic effect), anti-tumour action (IR radiation capable of inhibiting the proliferation of cancer cells and enhances chemotherapy efficacy), brain neuroprotection (treatments for stroke, traumatic brain injury (TBI) in vivo models (Naeser et al. 2011, Salehpour et al. 2017) and neurodegenerative disorders for Alzheimer’s and Parkinson’s diseases. These studies have been summarized in Table 1. Therefore, a better understanding of the mechanisms using IR radiation, could support improved therapeutic effectiveness via new strategies of PBM therapy at IR wavelengths (Tsai and Hamblin 2017).

Laser is a device, which produces intense, monochromatic, coherent, and highly collimated beam of light (Fonseca et al. 2010). Laser light has quite pure frequency, which makes it useful for biomedical applications (Ratkay-Traub et al. 2001). 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 (Hawkins et al. 2005). 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 (Hawkins and Abrahamse 2006). Class I, which refers to 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 ($10^{-4}$ to $10^{0}$ W/cm$^2$) and a dose of $10^{-2}$ to $10^{2}$ J/cm$^2$ (Posten et al. 2005).

However, 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 (Fonseca et al. 2010; da Silva Sergio et al. 2012); and for medical treatment (Vasheghani et al. 2009; Ahraei et al. 2014; de Menezes et al. 2015; Bayat et al. 2016). 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 (b) 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 (Hashmi et al. 2010b). Therapeutically, the super-pulsed GaAs and In-Ga-As lasers are capable of deep penetration without the undesirable influences associated with continuous 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 a well accepted tool in medicine and dentistry (Amid et al. 2014, Table 2). It is known by its ability to incite a thermic, non-damaging photobiological action (McDaniel 2015). Unlike ‘hard’ high power lasers, LLLT provides low energy, only sufficient to induce stimulation response of body tissue. It has a wavelength-dependent manner able to change the cellular function, in the absence of significant heating (Surendranath and Arjun 2013). Hence, LLLT is also called ‘soft’ laser therapy or cold laser, as a low-energy laser has no thermal effects (Nelson 1993; Chung et al. 2012).

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 (Chung et al. 2012). To produce photo-biological action, photon absorption of laser radiation must occur (Hawkins et al. 2005). Endogenous or exogenous chromophores are the initial photoacceptor molecules (i.e. molecules that can absorb light at certain wavelengths) that are able to absorb the incident photon energy (Bjordal et al. 2001). A photochemical conversion of the photon energy absorbed by a photoacceptor has been demonstrated (Brondon et al. 2005). 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 (Mochizuki-Oda et al. 2002; Brondon et al. 2005). 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 (Karu 1999).

Several studies suggested that mitochondria is the most sensitive component of cell to visible and near infrared light (Karu 1999; Karu et al. 2001) 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 (Hamblin and Demidova 2006). Moreover, PBM at red and NIR wavelengths stimulate increasing intracellular calcium Ca$^{2+}$ (Irvine and Schell 2001; Santana-Blank et al. 2005; Karu 2008; de Freitas and Hamblin 2016), 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 (Wang et al. 2016). Many researchers suggested that the response of
| Study No. | Type of laser | Wavelength (nm) | Power (mW) | Energy density (J/cm²) | Power density (mW/cm²) | Emission model | Types of diseases | Reference |
|----------|---------------|-----------------|------------|------------------------|------------------------|---------------|-------------------|-----------|
| 1        | Diode laser   | 810             | 10         | 3 and 30               | 5 and 50               | CW            | Zymosan-induced arthritis | (Castano et al. 2007) |
| 2        | He-Ne         | 632.8           | 10         | 3, 5, 10, 20, 25 and 50 | 64.6                   | CW            | Neurodegenerative | (Song et al. 2012) |
| 3        | He-Ne         | 632.8           | 10         | 0.5, 1, 2 and 4        |                        | CW            | Alzheimer's disease | (Meng et al. 2013) |
| 4        | Nd:YAG        | 1064            | 1.25       |                        |                        | CW            | Dental/Tooth extraction | (Vescovi et al. 2013) |
| 5        | GaAs          | 904             | 10         | 5.4                    | 20                     | CW            | Musculoskeletal diseases | (Bjordal et al. 2006) |
| 6        | Diode laser   | 830             | 30         | 1.1                    | 30                     | Pulse         | Painful stomatitis control | (Toida et al. 2003) |
| 7        | Diode laser   | 810             | 30         | 0.9                    |                        | CW            | Diabetic wounds        | (Danciókó et al. 2014) |
| 8        | Diode laser   | 830             | 30         |                        |                        | CW            | Chronic diseases of inner ear | (Wilden and Dindinger 1996) |
| 9        | He-Ne         | 632.8           | 50         | 2                      |                        | CW            | Chronic lichenoid graft-vs.-host disease (cGVHD) | (Chor et al. 2004) |
| 10       | Diode laser   | 810             | 3          | 20                     |                        | CW            | Cortical neurons        | (Huang et al. 2014) |
| 11       | He-Ne         | 632.8           | 300        | 3                      | 3000                   | Pulse         | Alzheimer's Disease     | (Farfara et al. 2015) |
| 12       | GaAlAs        | 860             | 30         | 3                      |                        | CW            | Osteoarthritic (OA) pain | (Brosseau et al. 2005a,b) |
| 13       | GaAs          | 808             | 10         | 45                     | 4000                   | CW            | Traumatic brain injury (TBI) | (Kabir et al. 2007) |
| 14       | GaAlAs        | 830             | 60         | 6                      |                        | CW            | Lumbago                | (Ohshiro and Shibata 1992) |
| 15       | Diode laser   | 660             | 30         | 7.5                    |                        | CW            | Lung neutrophils        | (Aimbre et al. 2008) |
| 16       | Diode laser   | 660             | 40         | 20                     |                        | CW            | Burning mouth syndrome  | (dos Santos Lde et al. 2011) |
| 17       | Diode laser   | 665, 730, 810   | 36         | 150                    |                        | CW            | Traumatic brain injury (TBI) | (Wu et al. 2012a) |
| 18       | Diode laser   | 660             | 24         |                        |                        | CW            | Periodontal disease     | (de Almeida et al. 2008) |
| 19       | Diode laser   | 820             | 300        | 3                      |                        | CW            | myofascial pain (MP) dysfunction syndrome | (Öz et al. 2010) |
| 20       | GaAlAs        | 780             | 50         | 7.5                    |                        | CW            | Rheumatoid arthritis     | (Ekim et al. 2007) |
| 21       | Diode laser   | 810             | 70         | 0.03, 0.3, 3, 10 and 30 | 25                     | CW            | Cortical neurons         | (Sharma et al. 2011) |
| 22       | GaAlAs        | 810             | 1 W        | 4.8                    | 80                     | CW            | Orofacial nerves regeneration | (Midamba and Haanen 1993) |
| 23       | GaAs          | 830             | 70         | 6                      |                        | CW            | Chronic periodontitis    | (Makhlouf et al. 2012) |
| 24       | Diode laser   | 830             | 100        | 3                      |                        | CW            | Temporomandibular joint pain | (Chang et al. 2014) |
| 25       | Diode laser   | 780             | 30         | 6.3                    |                        | CW            | Temporomandibular joint pain | (Chang et al. 2014) |
| 26       | He-Ne         | 632.8           | 10         | 0.18–27                |                        | CW            | Indolent ulcers          | (Schindl et al. 1992) |
| 27       | Diode laser   | 808             | 300        | 110                    | 165                    | CW            | Hearing loss             | (Tamura et al. 2015) |
| 28       | Diode laser   | 532             | 7.5        |                        |                        | CW            | Hearing loss             | (Goodman et al. 2013) |
| 29       | Diode laser   | 635             | 5          |                        |                        | CW            | Complaints of Tinnitus   | (Salahadlin et al. 2012) |
| 30       | InGaAs        | 660             | 10         | 2.5                    |                        | CW            | Acute zymosan-induced arthritis | (Carlos et al. 2014) |
| 31       | GaAs          | 904             | 20         | 2–20                   | 11.2                   | Pulse         | Chronic myofascial pain syndrome (MPS) in the neck | (Gur et al. 2004) |
| 32       | GaAs          | 904             | 29.5       |                        | 246                    | Pulse         | Salivary glands (keratonia) | (Lončar et al. 2011) |
| 33       | Diode laser   | 630–670         | 10–100     | 2, 3 and 4             |                        | Pulse         | Oral mucositis due to cancer therapy | (Bensdoun and Nair 2012) |
| 34       | Diode laser   | 660, 810 and 980| 780–830    | 36                     |                        | CW            | Traumatic brain injury (TBI) | (Wu et al. 2010) |
| 35       | GaAlAs        | 670             | 5          | 2                      |                        | CW            | Chronic periodontitis     | (Obradovic et al. 2013) |
| 36       | GaAs          | 780             | 22         | 7.7                    | 100                    | CW            | Diabetes mellitus (DM)    | (Narva et al. 2013) |
| 37       | Diode laser   | 810             | 36         | 50                     |                        | CW            | Traumatic brain injury (TBI) | (Xuan et al. 2015) |
|   | Laser Type | Wavelength (nm) | Power (mW) | Duty Cycle (%) | Modulation (Hz) | Application | Reference |
|---|------------|-----------------|------------|----------------|-----------------|-------------|-----------|
| 38 | Diode laser | 685 | 200 | 2 | CW | Reynaud’s phenomenon | (Hirschl et al. 2004) |
| 39 | Diode laser | 810 | 50 | CW | Parkinson’s disease (PD) | (Trimmer et al. 2009) |
| 40 | Diode laser | 790 | 120 | 6 | CW | Burning mouth syndrome | (Kato et al. 2010) |
| 41 | IR laser | 830 | 35 | 3 | CW | Lung inflammation | (Oliveira et al. 2013) |
| 42 | GaAs | 904 | 150 | 6 | Pulse | Carpal tunnel syndrome | (Dakowicz et al. 2011) |
| 43 | AlGaAs | 780 | 30 | 22.5 | 750 | CW | Renal interstitial fibrosis | (Oliveira et al. 2012) |
| 44 | GaAlAs | 830 | 60 | 18 | 3000 | Pulse | Knee osteoarthrosis | (Trelles et al. 1991) |
| 45 | AlGaAs | 785 | 70 | 3 | CW | Rheumatoid arthritis | (Meireles et al. 2010) |
| 46 | Diode laser | 670 | 50 | 3 | Pulse | Temporomandibular disorder (TMD) | (Núñez et al. 2006) |
| 47 | GaAs | 904 | 45 | 5 | CW | Muscle trauma | (Rizzi et al. 2006) |
| 48 | GaAlAs | 980 | 300 | 4 | 1500 | CW | Muscous membrane | (Cafaro et al. 2012) |
| 49 | Diode laser | 660 | 5 | 4.5 | CW | Acute lung inflammation | (de Lima et al. 2011) |
| 50 | GaAs | 980 | 10 | 2-4 | Pulse | Chronic low back pain (LBP) | (Hadi et al. 2009) |
| 51 | GaAlAs | 980 | 300 | 4 | 1000 | CW | Oral lichen planus | (Cafaro et al. 2014) |
| 52 | GaAs | 660 | 30 | 57.14 | 428 | CW | Periodontal disease (PD) | (Garcia et al. 2011) |
| 53 | InGaNP | 660 | 40 | 2 | 1000 | CW | Ulcers in patients with leprosy sequelae | (Barreto and Salgado, 2010) |
| 54 | GaAlAs | 815 | 250 | 12 | CW | Inflammation in retrodiscal tissues in patients with temporomandibular joint | (Kucuk et al. 2010) |
| 55 | GaAlAs | 808 | 500 | 5 | 1.8 | CW | Bisphosphonate related osteonecrosis of jaws | (Altay et al. 2014) |
| 56 | AsGaInP | 660 | 50 | 12.5 | 1.25 | CW | Third-degree burns | (Brassoletti et al. 2016) |
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 (Kulbacka et al. 2017; Roska and Juettner 2017; Roska and Lagali 2018). 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 (Schneider et al. 2015). The precise mechanism of laser-tissue interaction has not been completely explained, thus restricting the means to offer a clinical treatment protocol at present (Amid et al. 2013).

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 summarized 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.

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) (Basso et al. 2013). At the cellular and molecular level, there is still significant argument regarding the effectiveness of lasers in producing the desired practical responses (Basso et al. 2013). 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 (Koutná et al. 2003). The beam energy of laser light is powerful because it is highly coherent (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 (Koutná et al. 2003). 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 (Thompson 1988).

Laser light is characterised by its single wavelength, although some lasers, such as the dye laser, can be tuned over a wide range of wavelengths (Singh et al. 2012). 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 (Karu et al. 2004). In the medical field, lasers are classified as either high-power surgical lasers or low-power therapeutic lasers (Mbene 2008). 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 (Koutná et al. 2003). The wavelengths of laser radiation used have been investigated to show their therapeutic use (Smith 1991).

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 390–10,600 nm and output powers up to 500 mW (AlGhamdi et al. 2012). 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 (Smith 1991).

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 (AlGhamdi et al. 2012). 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 health care (Koutná et al. 2003, Table 1). 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 (AlGhamdi et al. 2012).

From observations, it appears that LLLT has beneficial effects at the molecular, cellular and tissue levels (Tafur and Mills 2008). It has been found that medical treatment with LLLT at various intensities has stimulatory effect on cellular processes (Avci et al. 2013). Recently, it has been reported

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### Table 2. Parameters involved in LLLT applications.

| Parameter                  | Unit of measurement | Definitions                                                                                                                                 |
|----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| Wavelength                 | nm                  | An electromagnetic radiation travels in discrete packets that also have a wave-like property. It is the amount of energy consumed per unit time, and can be calculated as: Power (P) = Energy (J)/Time (sec) |
| Power                      | W                   | 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 (W)/Area (cm²) |
| Power density              | W/cm²               | It is the allowed interval through which the energy has delivered to the target system.                                                   |
| Energy density             | J/cm²               | An electromagnetic radiation travels in discrete packets that also have a wave-like property. It is the amount of energy consumed per unit time, and can be calculated as: Power (P) = Energy (J)/Time (sec) |
| Total irradiation time     | sec                 | It is the allowed interval through which the energy has delivered to the target system.                                                   |

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Table 2. Parameters involved in LLLT applications.
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 |
|----|----------------|-------------------------|--------------|----------------------|---------------------------------|
| 1) | Human skin fibroblast cells | Cultures in minimum essential medium with Ear's balanced salt solution and incubated at 37°C in 5% CO₂ and 85% humidity | He-Ne Laser | Energy density (ED): 5 J/cm² | 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 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 A549 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. |
| 2) | E. Coli AB1157, BW951, BW991, and BW375 | Cultures in exponential and stationary growth phase. E. coli suspensions (1–2 × 10⁸ cells/mL in 0.9% NaCl solution) | Laser HTM compact model, AlGaInP | Power: 10 mW, Energy density: 0.658 nm | 1) There is no alteration of survival fractions of these E. coli cultures when exposed to laser.  
2) Laser exposure, at all emission modes, induced filamentation in exponential E. coli cultures at all emission modes.  
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.  
4) LLLT can increase the proliferation rate of various E. coli cell cultures when exposed to laser.  
5) The stimulation of cellular proliferation rate of lower doses of laser irradiation, at lower doses increase the cell proliferation rate and other cellular functions, while higher doses have negative effects. |
| 3) | Stem cells | Does not maintain the culture procedure | He-Ne Laser: Gallium-Aluminum-Arsenide (Ga-Al-As) | Power: 1–500 mW, Energy density: 0.5–4.0 J/cm² | 1) LLLT can increase enhance the proliferation rate of various cell lines.  
2) The stimulation of cellular proliferation rate of lower doses of laser irradiation, at lower doses increase the cell proliferation rate and other cellular functions, while higher doses have negative effects. |
| No | Cell types used                             | How the cells are grown                                                                 | Type of LLLT          | Quality of laser used                                                                 | Biological effects determination                                                                 | References          |
|----|--------------------------------------------|----------------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------|
| 4) | Mesenchymal stem cells (MSCs) and Cardiac stem cells (CSCs) | Cell cultured at 1.3 × 10⁶ cm² 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 streptomycin | Diode (Ga-As)        | 2. 804 nm Power density: 50 mW/cm²  
Energy density: 1 and 3 J/cm²  
Exposure time: 20 sec or 60 sec | 1) CSCs of (1 J/cm²) 1 and 2 weeks post LLLT irradiation significant increase of seven-fold and two-fold, respectively in the number of 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 MSCs increased post LLLT of 50 mW/cm² for 20 sec and 60 sec | (Tuby et al. 2007) |
| 5) | Fibroblast of skin cells, buccal mucosa and gingival | CSC cultured in a class 2 flow hood.                                                  | Diode (Ga–Al–As) diode laser | 2. 810 nm Power: 50 mW  
Energy density: 4 J/cm²  
Exposure time: 32 sec | 1) Increased proliferation, maturation and locomotion as well as transformation to myo-fibroblasts.  
2) Reduced production of pro-inflammatory prostagland in E2  
3) Increased production of basic fibroblasts growth factors.  
4) Increased proliferation at low doses and suppressed at high doses.  
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. | (Walsh et al. 1997) |
|    | Macrophages                                 |                                                                                        |                       |                                                                                        |                                                                                                   |                     |
|    | Lymphocytes                                |                                                                                        |                       |                                                                                        |                                                                                                   |                     |
|    | Epithelial cells                           |                                                                                        |                       |                                                                                        |                                                                                                   |                     |
|    | Endothelium cells                          |                                                                                        |                       |                                                                                        |                                                                                                   |                     |
| 6) | Human Gingival Fibroblasts (Hgf3-Pi S3 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, 100U/ml pencillin, and 100 µg/ml streptomycin. | Diode (Ga–Al–As) diode laser | 2. 810 nm Power: 50 mW  
Energy density: 4 J/cm²  
Exposure time: 32 sec | 1) The differences between the case and the control groups were statistically significant on 48h and 72h 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. 2013) |
| No. | Organ/Cell Type | Description | Laser/光源 | Wavelength ($\lambda$) | Power | Diameter of Beam | Energy Density | Exposure Time | Notes |
|-----|----------------|-------------|------------|-----------------------|------|-----------------|--------------|--------------|-------|
| 7)  | HeLa cells     | They were grown as monolayers in scintillation vials | He-Ne laser | $\lambda$: 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 $\gamma$-radiation, substantial differences was seen between the survival curve and the curve representing the survival of $\gamma$-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. 1994) |
| 8)  | Yeast, HeLa    | He-Ne laser | $\lambda$: 632.8 nm | Power density: $I \geq 2 \times 10^{11}$ 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 1988) |
| 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% CO$_2$ atmosphere. The cells were sub-cultured twice weekly in fresh RPMI 1640 medium. | He-Ne laser | $\lambda$: 632.8 nm | Power: 10 W | Diameter of beam: 0.75 cm | 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. 2001) |
| 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-glucero-phosphate (Sigma) | GaAlAs diode laser | $\lambda$: 780 nm | Power: 70 mW | 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 (Petri et al. 2010) |
| 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 | $\lambda$: 670 nm, 780 nm, 692 nm, 786 nm | 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. (Almeida-Lopes et al. 2001) |

(continued)
| No | Cell types used       | How the cells are grown                                                                 | Type of LLLT     | Quality of laser used                                                                 | Biological effects determination                                                                 | References         |
|----|-----------------------|--------------------------------------------------------------------------------------------|------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------------|
| 12 | Human Macrophages     | The macrophage J774 cell line was grown in (DMEM) supplemented with 10% 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 h using an inverted phase microscope. | Diode laser      | λ: 780 nm  
Power: 70 mW  
Energy density: 3 J/cm²  
λ: 660 nm  
Power: 15 mW  
Energy density: 7.5 J/cm² | 3) Lasers of equal output power presented the similar effect on cell growth independently of their wavelength.  
1) After 1 day of culture, activated and 780 nm irradiated macrophages showed lower mitochondrial activity (MA) than 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.  
Pulsed low-level laser with low-energy density range appears to exert a bio-stimulatory effect on bone tissue. | (Souza et al. 2014) |
| 13 | MG-63                 | Cells were maintained 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°C in a humidified atmosphere of 95% air and 5% CO₂. | Diode laser      | λ: 940 nm  
Energy outputs: 1–5 J  
Intensities: 0.5, 1, 1.5 and 2 W/cm² | Reduction in cell proliferation compared to non-irradiated controls. | (Huertas et al. 2013) |
| 14 | Osteoblastic (MC3T3) cell line | Cells were grown in sterile Dulbecco’s Modified Eagle’s Medium: Nutrient Mixture 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: 30 mW  
Energy density: 10 J/cm² |                                                                                       | (Renno et al. 2010) |
| No. | Stem Cells Type                          | Growth Conditions                                                                 | Laser Parameters | Effect | Ref. |
|-----|-----------------------------------------|-----------------------------------------------------------------------------------|------------------|--------|------|
| 15) | Human osteoblast cell line              | Cells were maintained in sterile medium: Dulbecco’s Modified Eagle’s Medium: Nutrient Mixture F-12 (DME = 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 $\lambda$: 632 nm Power: 10 mW Energy density: 0.43 J/cm² | LLLT promotes proliferation and maturation of human osteoblasts in vitro, and a significant 31–58% increase in cell survival | (Stein et al. 2005) |
| 16) | Human monocytic THP-1 cell line         | THP-1 cells were grown in 50 ml culture flask, the flask containing 20 ml of medium plus cell, at 37°C with 9% CO₂ in a humidified incubator. | Diode laser 850 nm $\lambda$: 850 nm Power: 9.5 mW Energy density: (0.6–27 J/cm²) power density of 29.6 mW/cm² | PBM promotes proliferation of human monocyte in vitro, and a significantly increased cell survival due to increasing membrane integrity and mitochondrial activity. | (Musstraf et al. 2017) |
| 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 $\lambda$: 660 nm Energy density: (1.2–6.2 J/cm²) | Improved cell viability and proliferation of SHED after laser irradiation, except for 1.2 J cm⁻². | (de Souza et al. 2018) |
by several investigators that at low levels of red or near-infra-red light illumination, LLLT can prevent cell apoptosis (Huang et al. 2009; AlGhamdi et al. 2012), stimulation of mitochondrial activity, increased cell turnover, recruitment and proliferation, modulation of the cellular metabolites (Di Giacomo et al. 2013). It was suggested that LLLT might promote changes in the cellular redox state, playing an important role in sustaining cellular activities and induce photobiostimulative processes (Silveira et al. 2009). In addition to the above, pre-exposure of PBM had a protective effect against many external agents such as hydrogen peroxide (H₂O₂) and UV radiation (Canuto et al. 2015; Sergio et al. 2015). There is an evolutionary standpoint confirming that NIR pre-exposure can protect cells from the hazards of UV exposure and that re-exposure for NIR radiation could be important for protection maintenance (Continenza et al. 1993; Lettini et al. 2016).

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:

\[
\sin \theta_1 \sin \theta_2 = n_2/n_1
\]

Where \( \theta_1 \) is the angle between the incident light and the surface normal in the air, \( \theta_2 \) 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 (Niemz 2007).

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 the key for the desired impact on tissue healing. The magnitude of optical absorption is described in terms of the absorption coefficient \( \mu_a \) in units of cm⁻¹ (Jacques 2013). The depth of penetration (mean free path) into the absorbing medium is defined by the inverse, \( I_0 \) (Chung et al. 2012).

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 \( \mu_s \) (cm⁻¹) (Palan 2007; Niemz 2007). 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, \( \mu'_s \) (cm⁻¹), which can be defined as:

\[
\mu'_s = \mu_s(1 - g)
\]

The sum of absorption coefficient (\( \mu_a \)) and scattering coefficient (\( \mu_s \)) 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, \( \mu \) (cm⁻¹):

\[
\mu = \mu_s + \mu_a
\]

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 (Chandrasekhar 1960) and 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, scattering processes and emission, (Chandrasekhar 1960, Lenoble 1985). 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:

\[
s \cdot \nabla L(r, s) = -(\mu_a + \mu_s)L(r, s) + \mu_s \int p(s, s') L(r, s')d\omega'
\]

Where \( d\omega' \) is the differential solid angle in the direction \( s' \), and \( p(s, s') \) is the phase function (Cheong et al. 1990; Chung et al. 2012).

Determining the distribution of light in an irradiated tissue is based on the transport equation requiring \( \mu_a, \mu_s \), 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) (Cheong et al. 1990).

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 (Hashmi et al. 2010a; Di Giacomo et al. 2013). 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 (Huang et al. 2009). A chromophore (or
When photons from a laser are incident on living tissue, scattered photons are reflected or transmitted (Hamblin and Demidova 2006). Absorbed photons interact with chromophore molecules 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 (Smith 2005). 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.

### 4.1. Pathway 1

This is the most common pathway that occurs and is called internal conversion, the excited singlet state of a chromophore is transported from a higher to a lower electronic state. This transition takes place without photons emitting, known as non-radiative decay (Hamblin and Demidova 2006). 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 excitation energy is transformed into heat. This process would not be expected to cause chemical changes to the molecule (Smith 1991).

### 4.2. 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) (Smith 2005). The resultant heat (from molecular vibrations) arises from the energy difference between the absorbed and emitted photons.

### 4.3. 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 (Hamblin and Demidova 2006).

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 colour 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 (Hamblin and Demidova 2006). 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 (Silveira et al. 2009). It is also considered as the photosignal transducer in the region of visible and IR-A region (Karu 2010). This reactivity is due to four redox active metal centres: the bi-nuclear CuA, CuB, heme a and heme a3, all of which have strong absorbency in the red to IR-A range (Smith 2007; Karu 2010; Piazena and Kelleher 2010).

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) (Smith 2005; Desmet et al. 2006).

Cytochrome c oxidase is the fourth enzyme in the inner membrane of cellular mitochondria (Habash et al. 2006; Huang et al. 2011; Di Giacomo et al. 2013), that plays a pivotal role in adenosine tri phosphate (ATP) synthesis (Silveira et al. 2009). 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 (Silveira et al. 2009). Moreover, signal transduction to other parts of the cell has occurred, including cell membranes (Woodruff et al. 2004). Photobiological responses are the result of photochemical and/or photophysical changes after the absorption of non-ionizing electromagnetic radiation (Smith 1991).

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 (Brown 1995). This binding is proposed to dissociate by the PBM or LLLT effect and reverse the mitochondrial inhibition of respiration due to excessive NO binding (Lane 2006). 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 species (RNS) (Alexandratou et al. 2002; Lavi et al. 2003; Lubart et al. 2005; Zhang et al. 2008; Cotler et al. 2015). The excited
mitochondrial cytochrome c oxidase after absorbing NIR radiation photon generates ROS that causes changing the oxidation state of the mitochondrial membrane (Gilmore 2006).

For the phototherapeutic effect to be observed, the appropriate wavelength of light and dose (fluency) of radiation are needed (Woodruff et al. 2004). However, phototherapy will not be effective on every system and in every situation. It has been emphasised that the magnitude of the phototherapy effect depends on the physiological state of the cell at the time of irradiation (Karu 1989).

5. Light emitting diodes (LEDs)

A light emitting diode (LED) is a semiconductor light source (Han et al. 2011). 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 (Desmet et al. 2006; Suparman 2013). A LED is formed by p-n junctions (p-positive, n-negative), but not all semiconductors are suitable for use as LEDs (de Abreu Chaves et al. 2014). The physical mechanism by which LED emits light is spontaneous emission (de Abreu Chaves et al. 2014). They emit near-monochromatic, incoherent light (Ishida 2005), in a process called electroluminescence (Molinarioli 2001). 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 (Ishida 2005), with a linewidth of around 10–30 nm. LEDs have been publicised as a comfortable, potentially highly selective light-based therapies for many indications (McDaniel et al. 2003). LEDs are also very controllable as light sources for non-thermal applications, acquiring a broad area of use in medical applications (Avci et al. 2013).

5.1. Laser light vs. light emitting diode (LED)

Not all light is the same or has equal medical benefits (LED or LASER therapy). Recently, controversy has arisen around the comparison between low level laser therapy and light emitting diodes, which have completely different biological effects (Agnol et al. 2009). A number of studies have been published to determine the effectiveness of LLLT to LED light (Figure 1). The majority of the studies have found that although lasers have small focused spots, so only a small area of tissue (<1 cm²) is exposed to light; whereas LEDs usually have a large area (100 cm²), so much more area of tissue is exposed to light, lasers are far more effective (Agnol et al. 2009). Laser therapy can achieve much greater and deeper stimulative and therapeutically beneficial effects. Laser beams are easily manipulated using Gaussian beam optics, a simple analytical tool, to enable a laser beam to be fully controlled spatially, position, size, etc. As an LED is difficult to control in terms of position and spot size, it is limited for treatment of superficial tissue only. However, LED light has some beneficial effect where it is believed that it can have a photo-modulation effect on certain cellular and sub-cellular receptors. In addition, they have greater choice of wavelengths, are low cost and suitable for acute and chronic conditions (Darren Starwynn 2004). In Figure 1, it can be seen that there is a significant increase in the applications of laser sources with wavelengths of greater than 800 nm. Why should this be when there are no known new photoreceptors reported in recent years? This could be attributed to technological advances and differences in the way lasers and LEDs can be controlled. LED sources are limited by the fundamental properties of silicon, which means that their spectral bandwidth has an upper bound of around 950 nm. In addition, LEDs can be operated in continuous or modulated modes only. For a comparative analysis, laser sources will also be restricted to laser diodes.

Figure 1. The number of papers published using lasers and LEDs sources in clinical and laboratory studies on the effect of LLLT from 1965–2018.
When light is incident on any material, in this case tissue, it will penetrate some distance into the tissue and this distance increases at longer wavelengths. The most significant factor in the application of lasers compared to LEDs is their mode of operation. Laser sources can be driven in pulsed mode, which can deliver a stream of pulses where the power in each pulse is determined by the pulse width in ns. Thus, lasers are able to efficiently deliver energy to cells and tissues, whereas this is impossible with LEDs (Moskvin 2017; Sato et al. 2016). LEDs still have important applications for photobiomodulation, but coherent sources operated in pulsed mode are still able to deliver light in more controlled ways deeper into tissue compared to modulated incoherent sources (Hamblin 2016).

A number of studies have been published comparing these two modalities. Kubota and Ohshiro (2004) treated rat skin flaps with an 830 nm GaAlAs laser and an 840 nm infrared LED. They found an increasing flap survival area in a rat model after being irradiated with 830 nm laser. Flaps treated with the laser had better perfusion, a greater number of larger blood vessels and significantly enhanced flow rates, while flaps treated with an 840 nm IR LED showed no difference from the control group (Kubota and Ohshiro 2004). Berki et al. (1988) used a HeNe laser to stimulate cell activation in vitro. They observed increasing phagocytic activity along with immunoglobulin secretion, but this effect was not seen after irradiation of the cell cultures with LED light of the same wavelength and doses (Berki et al. 1988).

A comparative study has been performed by Haina et al. (1982) to show the effectiveness of a coherent HeNe 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 (Haina et al. 1982). Rockhind et al. (1989) 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 (Rochkind et al. 1989). Laasko et al. treated patients with chronic pain using an 820 nm IR laser at 25 mW, a 670 nm laser at 10 mW and a 660 nm LED (Laasko et al. 1994). They found an elevated level of ACTH and beta endorphin in the laser therapy groups but not in the LED group (Laasko et al. 1994).

The effect of HeNe laser and incoherent LED light on leukocytes in migration inhibition assays has been studied by Lederer et al. (1982). They reported that irradiation with HeNe laser light affected leukocytes. However, incoherent light of the same wavelength and power density showed no influence (Lederer et al. 1982). Al (1989) investigated the role of coherent laser therapy in wound healing. They noticed that HeNe lasers with a dose of 1 J/cm² produced an acceleration of the healing process, but incoherent light of the same wavelength and dose was less favourable (Al 1989).

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 near-infrared light emitted by these LEDs seems to be perfect for increasing energy inside cells. This means whether you are 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' (Somer 2001; Darren Starwynn 2004). Oliveira Sampaio and colleagues (2013) studied the effect of low-level light therapy on the healing of cutaneous wounds 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 anemic animals. However, laser light was more effective in increasing proliferation on non-anemics (Oliveira Sampaio et al. 2013). A clinical study by Esper and Arisawa (2011) 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 (Esper and Arisawa 2011).

Agnol et al. (2009) 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 (Agnol et al. 2009). Similar findings have been obtained by Klebanov et al. (2005) in a comparative study of the effect of laser and light emitting diode irradiation on healing and functional activity of wound exudate leukocytes (Klebanov et al. 2005). 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 (Klebanov et al. 2005). Another study by Klebanov et al. (2006) has been carried out to explore the comparative effects of laser light and light emitting diodes on the production of superoxide dismutase (SOD) and nitric oxide (NO) in wound fluid of rats. The study indicated dose-dependent changes in superoxide dismutase activity and production of nitrates 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 nitric oxide formation (Klebanov et al. 2006).

The rapid evolution of light emitting diodes makes feasible the use of LEDs for medical treatment and light therapy (Yeh...
et al. 2010). The single frequency laser does not diffuse, whereas the LED light does. This diffusion allows the cell to be in control of the treatment (Ghuloom 2013). Moreover, LED light therapy has been considered non-significant risk by the FDA (Desmet et al. 2006). For this reason, it was suggested that the use of light emitting diodes for treatment is much safer than laser therapy (Ghuloom 2013).

Given the above information, and from recently published studies (Lee et al. 2007; Oliveira et al. 2013), it has been shown that lasers have an important role in many medical conditions and with many positive research results (Avci et al. 2014; Cotler et al. 2015; Bell and Stout 2018), as well as LEDs, which are also important in many cases of the disease (Corazza et al. 2007; Xavier et al. 2010). Nevertheless, in most comparative studies that used laser and LED with the same qualities (wavelength, doses, intensity), it is confirmed that laser offer many advantages compared to LEDs (Leal Junior et al. 2009).

Given that LEDs are relatively cheaper and easier to use, it is not surprising that compared to lasers, the number of articles using LEDs for clinical applications are on the increase. There are, however, only a few studies where biological responses of these two types of sources have been compared concomitantly in the same biological model. This is, particularly so, at molecular and cellular levels with clinical implications or outcomes. In a comprehensive study, using various in vitro models derived from (a) 3T3 cells (standard fibroblast) stably expressing red fluorescence protein (DsRed), (b) EGFR expressing A431 cells (established from skin carcinoma) and, (c) stably luciferase-transfected 468-luc cells (originating from pleural effusion of mammary gland and breast tissue), the relative biological responses of these two sources have been compared (Sato et al. 2016). The study suggested no significant differences (in terms of cellular swelling, bleb formation) in non-EGFR-expressing 3T3 cells after irradiation with either sources. The cytotoxicity based on luciferase activity in 468-luc cells, however, showed a significant decrease of relative light units (RLU) related to near infrared photoimmunotherapy (NIR-PIT) induced reductions in ATP production and significant differences between the two sources, laser being more cytotoxic at the same energy level than LED (Sato et al. 2016). This enhanced cytotoxicity (determined by LDH cytotoxicity assay) by laser was further confirmed in spheroid (3D) cultures derived from A431 cells.

The in vitro studies by Sato et al. (2016) was further extended to in vivo mice models. Tumour volume was found to be reduced significantly in the laser NIR-PIT group compared with the LED NIR-PIT group of A431 xenograft mice. Furthermore, after NIR-PIT, the tumour irradiated by laser demonstrated lower IR700 – fluorescence intensity than the tumour irradiated by LED. Overall, the results suggested that lasers result in higher efficacy under in vitro and in vivo conditions and thus has superior therapeutic tumour effects compared to LEDs at the same energy level. This has obvious implications for the choice of NIR-light source for human clinical trials.

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 (Figure 2). More recent studies have studied the bio-stimulatory effect of low level laser on cell proliferation processes.

Early work by Karu et al. (1994) 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 100 J/m². 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 was observed after stimulation with a He-Ne laser compared to the control group (Karu et al. 1994).

Pereira et al. (2002) examined a 632.8 nm He-Ne laser with an energy fluence of 0.053–1.89 J/cm² and a 904 nm (GaAs) laser with an energy fluence of 1.94 × 10⁻⁷ to 5.84 × 10⁻⁶ J/cm² 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 (Pereira et al. 2002).

It was noted that with exposure to a 670 nm GaAlAs laser, an increase in myofibroblasts and collagen deposition was observed (Madrado et al. 2003). Furthermore, an increase in gingival fibroblasts after exposure to diode lasers (670, 692, 780, and 786 nm) was also found (Posten et al. 2005).

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Bouma et al. (1996) examined human monocytes and human umbilical vein endothelial cells (HUVECs) with a 904 nm GaAs laser at 40.18 mW/cm² 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 (Bouma et al. 1996). Schindl et al. (2003) reported that HUVECs irradiated with a 670 nm diode laser with a dose of 2–8 J/cm² resulted an increase in the proliferation of these cells that is determined by using a haemocytometer (Schindl et al. 2003). An in vitro study by Hass et al. (1990) showed an increase in human keratinocytes mortality after exposure to He-Ne laser and found no change in proliferation or differentiation (Haas et al. 1990). In contrast, Grossman et al. (1998) observed an increase in proliferation rate of keratinocyte cells after exposure to a 780 nm continuous-wave diode laser with a dose from 0 to 3.6 J/cm² (Grossman et al. 1998).

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 et al. (2012) have examined stem cells following exposure to a...
He-Ne laser at 632.8 nm and a GaAlAs at 600 nm, with a range of energy densities (doses) from 0.5 to 4.0 J/cm² 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. In contrast, higher doses of low-level laser therapy have negative effects, significantly decreasing cell counts and the viability (AlGhamdi et al. 2012). Similar results have been obtained by Walsh and colleagues (1997), when they irradiated skin fibroblasts cells, buccal mucosa and gingival cells with semiconductor lasers at 540 nm and 600–900 nm with 0–56 J/cm² energy densities. Walsh noted increased cell proliferation at low doses, which was repressed at high doses. They also observed increased maturation and locomotion, transformation to myo-fibroblasts and increased production of basic fibroblasts growth factors.

Walsh (1997) used the same laser with the same energy densities to examine the effects on macrophage cells. A convergent result was observed, which included 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 lymphocyte cells showing activated cells with 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 et al. (2010) found that cell survival, 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 a power of 70 mW and energy density of 3 J/cm² (Petri et al. 2010). Recently, Forouzanfar (2014) has supported Petri’s results when examining human gingival fibroblasts with a Ga-Al-As diode laser at 810 nm, output power of 50 mW and energy density of 4 J/cm². 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 found a significant difference between the case and control groups on 48 and 72 h after irradiation (Forouzanfar 2014).

Tuby et al. (2007) 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/cm² and an output power of 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 irradiation of 1 J/cm² for 20 sec exposure and increased the number of MSCs and CSCs after 1 week post irradiation of 3 J/cm² compared to the control (Tuby et al. 2007).

Almeida-Lopes et al. (2001) used diode laser with 670, 692, 780, and 786 nm wavelengths and fluence (energy density) of 2 J/cm² 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 independent of their wavelengths (Almeida-Lopes et al. 2001).

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 (Mbene 2008). However, the possibility of induced DNA damage has now arisen; even though this damage could be repairable (Mbene 2008). 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 (Kujawa et al. 2004).

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 sunlight, it is assumed that the doses per area of LLLT are safe when corresponding to the DNA damaging effects of a few minutes sunlight (Kujawa et al. 2004). If such irradiation induces DNA breaks, these breaks are likely to be repaired immediately; otherwise unrepaird damage could lead to mutations consequently leading to development of cancer in the long run (Albertini et al. 2008).

Different studies in eukaryotic and prokaryotic cells have reported adverse effects on cells and DNA damage after exposure to low-power laser therapy (Kong et al. 2009) (Figure 3). Experimental data about the effect of these light sources with different power, wavelengths and emission modes on DNA are, however, limited (Karu 2010). A study by Zhang et al. (2003) using microarray technologies indicated that low-intensity laser exposure (red light) at therapeutic doses promote expression of DNA repair genes following induction of DNA lesions by free radicals (Zhang et al. 2003).

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 (Smith 1991). 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 (original) DNA. Mbene (2008) treated wounded human skin fibroblast cells by He-Ne laser with 5 J/cm² and 16 J/cm² doses. Irradiation with 5 J/cm² and 16 J/cm² showed insignificant change in DNA damage, as determined by alkaline comet assay, at 1 h when compared to their respective controls. However, a significant decrease in DNA damage at 24 h incubation due to the mechanism of DNA repair was shown (Mbene 2008).

Fonseca et al. (2010) irradiated E. coli cells with low-intensity (AlGaNp) red laser with a power of 10 mW and with different fluencies (1, 4, and 8 J/cm²). 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 (Fonseca et al. 2010).

A study by da Silva Sergio et al. (2012) 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 mechanisms. Moreover, it was found that the survival mechanism against harmful radiation could be activated or induced after irradiation with monochromatic red light (da Silva Sergio et al. 2012). Kohli et al. (2001) examined E. coli cells with a He-Ne laser at 632.8 nm. They observed that irradiation with low-level He-Ne lasers induces photolysase gene (phr) and DNA repair genes investigated by phr gene expression assay. The magnitude of induction relies on fluorescence 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 (Kohli et al. 2001).

Dube et al. (2001) studied the effect of He-Ne laser 632.8 nm pre-irradiation on UVA-induced DNA damage in human B-lymphoblast cell line, as measured by comet assay. They found a decrease in UVA-induced DNA damage. However, the control cells showed higher DNA damage, the same rate of DNA damage in He-Ne laser pre-irradiated cells. The results suggested 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 (Dube et al. 2001).

Dillenburg et al. (2014) triggered epithelial cells with laser phototherapy (LPT) of energy density 4 J/cm² and 20 J/cm². They observed that laser phototherapy at a low-energy density of 4 J/cm² did not induce DNA damage or genomic instability as determined by comet assay. Interestingly, a low energy of LPT induced nuclear influx of the BRCA1 protein, which is involved in DNA repair process. Importantly, these findings suggest that LPT of low dose induces a safe level of reactive oxygen species (ROS), which accelerate healing (Dillenburg et al. 2014).

Ridha et al. (2012) 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 induced enzymes involved in repair process (Ridha et al. 2012). More recently, Trajano et al. (Trajano et al. 2014) 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 (Trajano et al. 2014).

Although most of the aforementioned studies have shown the effect of LLLT on cell proliferation, conflicting results have been published. The studies also tried to explain the induction effect of LLLT on DNA repair mechanisms with varying 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 parallel with philosophical evolution towards minimally invasive laser therapies (Alam and Dover 2003). 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 (Hamblin and Demidova 2006). Experiments of measuring the temperature following LLLT exposure have shown that the immediate increase in temperature of the irradiated tissue is negligible (±1 °C) (Hrnjak et al. 1994). Many researchers emphasises that the temperature remained unchanged in suspensions of different cells through LLLT irradiation (Boulton and Marshall 1986; Quickenden and Danniels 1993). Studies by Schneede et al. (1988) suggested that the temperature could raise by less than 0.065 °C, during irradiation with laser of 40 mW/cm², they used a microthermal probe in a monolayer of cells to measure the temperature (Schneede et al. 1988).

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 (Smith 2005). However, many researchers have 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 articles in the literature on photo therapy in recent years using incoherent light sources, such as LEDs (Smith 2005).

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 (Smith 1991). These discrepancies may be attributed to a variance in study design, including the use of different lasers and inequalities in parameter selection. In addition, as indicated by Karu (1989), it may relate to the physiological state of the cell at the time of irradiation (Smith 1991). In general, for laser studies to be useful, all the characteristics of the light emitted from laser source or by LEDs must be specified (Smith 2005).

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 including 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 LEDs has the most beneficial therapeutic impacts on biological systems. A relative comparison of biological responses with potential clinical implications using different sources (i.e. laser and LEDs) in the same model system has been very limited, which needs further elaboration. Furthermore, 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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