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Topical Review

Light-based therapy on wound healing: a review

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Abstract

Wound healing is a complex matrix and overlapping process. In order to accelerate the healing process and minimize bacterial infection, light-based therapy was applied to stimulate bio-reaction to improve healing. The aim of this paper is to review the effects induced by light source (laser and incoherent light like LED) on different biological targets. The light-based therapy techniques were categorized according to the wavelength, energy density, type of irradiance and activity of tissues in the healing process. Out of 80 cases, 77% were animal studies, 5% were human studies and 18% were cell studies. Around 75% of light-based therapy has an advantage on tissue interaction and 25% has no effect or inhibition on the healing process. The appropriate dose appears to be between 1 and 5 J cm^{-2}. At shorter wavelength, photobiostimulation would be effective with a high frequently administrated low-energy dose. On the other hand, for longer wavelength it is the reverse, i.e., more effective with a low frequent treated schedule and a high-energy dose.

Keywords: wound healing, low-level laser therapy, biophotonic, photobiostimulatory

(Some figures may appear in colour only in the online journal)

1. Background

Wounds are divided into two types: internal and external. An internal wound is created due to circulation, neuropathy or medical illness. Skin is a protective barrier for isolation of the body and environment. Breaking the barrier allows the creation of an external wound such as an incision, trauma or burn [1]. The external wound can repair itself via the normal healing process. This involves four stages including hemostasis, inflammation, proliferation and remodeling [2–4]. The healing time depends on the size of the wound, but the healing rate is an independent process [5]. When the barrier protection is open and exposed to the environment, bacteria are allowed to build up and breeding occurs in the wound site. Clinical treatment is needed in this case. In conventional treatment, medicine is applied to the wound such as antibiotics, dressing and ointment to prevent bacterial infection, activate metabolism and reduce wound pain [6]. Nowadays, laser therapy is attracting research applying it to *in vitro* and *in vivo* targets. Laser therapy is a drug-free, extremely safe, easy to apply procedure and complements many traditional therapies. Hence, it is much better than conventional treatment. Most importantly, laser therapy can minimize the risk of bacterial infection and thus accelerate the healing process.

2. Low-level laser therapy (LLLT)

Laser therapy was first demonstrated by Endre Mester in 1967. The experiment in hair growth of mice revealed unexpected

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results that led towards a new discovery in using laser treatment [7]. Laser therapy involves the application of a low-power laser that is irradiated on a wound in order to stimulate the healing process. It is also referred to as low-level laser therapy (LLLT) [8, 9]. The important mechanism in laser therapy is photobiostimulation. LLLT is a biophotonic technique which stimulates the biological cell through the absorption of photons [10–12]. Photobiostimulation research has been reported since the introduction of lasers in the biological field and clinical applications [13–17]. Nevertheless, the exact mechanism of LLLT is not yet fully understood. Theoretically, the laser is absorbed by a light agent such as the mitochondria, hemoglobin and melanin. Once the tissue has absorbed the light, an electron in lower orbit will be excited and produce internal conversion energy. Inter-cellular communication is motivated by bio-reaction and restores the normal cell function [17]. Laser therapy reduces the inflammatory reactions, increases collagen deposition and induces greater proliferation [18, 19].

The effects of lasers will depend on parameters like power density and exposure time. The possible result might be to stimulate or inhibit the healing process [20]. Several studies have been carried out utilizing different types of lasers. The absorption relies on light properties such as monochromatism, non-coherence and polarization as well as depending also on the energy gap of the bio-molecule [21, 22]. The intensity of light also affects the outcome of the laser treatment. High power lasers are commonly used to cut through tissue. They are suitable in surgery for cutting or cauterizing. Exposure to a high-power laser for treatment has limitations, as over-exposure will cause disaster. Low-power lasers will stimulate tissue repair through a process of photobiostimulation. These low-level lasers do not have enough power to damage tissue, consequently a heating effect, damage to the skin and side effects may not occur [23]. Different parameters used in various studies raise a lot of complications and difficulties for comparison. Laser therapy has many biological effects either on *in vivo* or *in vitro* samples. Amongst them are accelerated tissue repair and cell growth, reduced fibrous tissue formation, anti-inflammation, analgesia, improved vascular activity and increased metabolic activity. The LLLT treatment has been accepted among health care practitioners. However, there is still a lack of documentation regarding the application of LLLT.

The aim of this work is to review the effects of LLLT and its consequences. The dependence on the energy density, types of irradiance and activities of tissue in the healing process after irradiance with LLLT will be discussed in detail. In general, typical medical lasers operate in the wavelength range between ultraviolet and infrared. The spectrum of ultraviolet radiation is in the range 180–400 nm. An excimer laser is an example of an ultraviolet laser. Excimer lasers normally have large beam spot size. This is a disadvantage in medical applications due to low power density. Furthermore, due to the shallow optical penetration depth, the excimer laser is normally used in photoablation to remove superficial surface tissue. This is the mechanism for eye surgery to repair astigmatism and myopia, keratomileusis, diabetic retinopathy and microbial keratitis [24–28]. On the other hand, for a long wavelength radiation, it is able to penetrate deeper into the tissue. Thus, the choice of a specific laser wavelength will depend on its penetration depth into the tissue. As the wavelength is increased further into the infrared region, light is absorbed more by water which limits its penetration into the desired tissue [29]. LLLT can be operated in continuous or pulse mode.

3. LLLT targets and laser sources

Various biological targets and laser sources have been reported for wound healing treatment. 77% of wound healing studies used animals as targets, 4.8% were performed on humans and 18% on cells. 87.5% of the animal studies used rats as experimental subjects. The wavelength of laser used in the treatment covered the range from visible (470 nm) to infrared. Nearly 91% of the irradiance is in the form of continuous wave (cw) and the other 9% in pulse mode. Some reports claimed that the laser irradiation might enhance, inhibit or have no effect on *in vivo* or *in vitro* targets. Around 75% of laser therapies have a positive effect on tissue interaction and 25% have no effect or inhibit the healing process.

The most popular wavelength in LLLT is 632.8 nm, followed by 670 nm (as shown in figure 1). Other lasers, including diode laser at 904 nm, CO_2 (10.6 μm), Nd:YAG (1.064 μm), and ND:YLF (1.047 μm), are commonly employed as a source of illumination. In other cases, a combination of two or more different wavelengths is also used for wound treatment. Almost 60% of the combination techniques comprised short wavelength (632.8 nm), and long wavelength (904 nm).

Energy (J) or energy density (J cm^{-2}) is often used as an important parameter to describe the LLLT performance. Energy density is expressed as follows [22]:

$$\text{Energy density}\,(\text{J cm}^{-2}) = \frac{\text{Output power}\,(\text{W}) \times \text{time}\,(\text{s})}{\text{Beam area}\,(\text{cm}^2)}. \quad (1)$$

The range of energy density normally applied for treatment varies from 0.1 to 140 J cm^{-2}. LLLT often operates within 1–5 J cm^{-2} and it occupies almost 60% in the case studied. The frequent doses are 1–4 J cm^{-2}. The high-energy dose is considered in the range of 15–20 J cm^{-2}.

Wound parameters are an important aspect to be considered in order to measure the progress of wound healing. This is the way to quantify and monitor the healing progression. There are several familiar variables to estimate the rate of healing, such as wound contraction size, histology, tensile strength, blood flow and scoring system. In this study, 58% of researchers used histology to establish a wound healing progress. A scoring system for the histological assessment of wound healing is commonly performed by edema, leucocytes, macrophages, granulation tissue, fibroblasts, collagen and epithelialization [30–39].

4. Types of light source

4.1. Visible violet–blue–green–yellow laser

The case studies in this spectral range are listed in table 1. The wavelengths covered are within the range 442–532 nm.

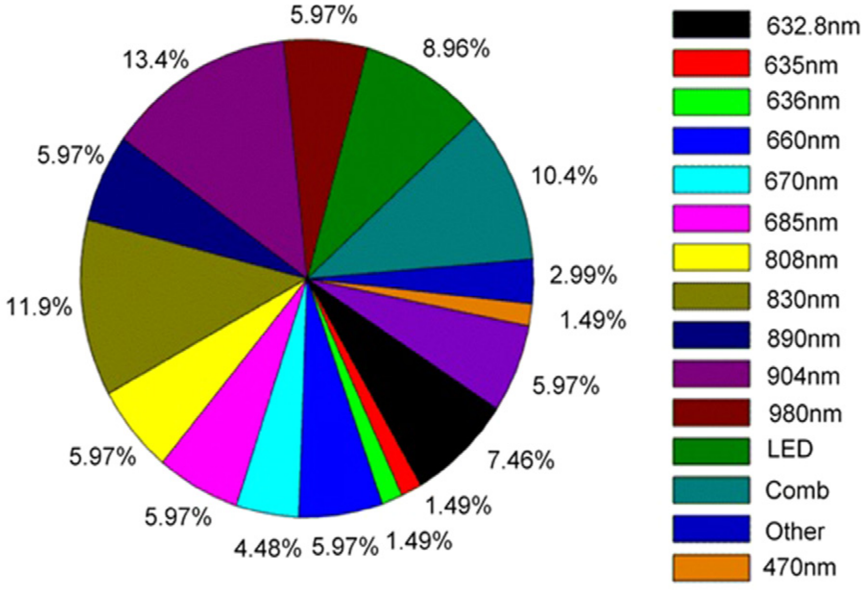

Figure 1. Percentage of tested wavelength for photobiostimulation in wound healing.

Table 1. Wound treatment with visible violet–blue–green–yellow laser.

Authors	Wavelength (nm)	Target	Energy or power density (J cm^{-2})	Outcome
Guffey *et al* [46]	405 470	*S. aureus* *P. aeruginosa*	1 3 5 10 15	405 nm effectively kill *S. aureus* and *P. aerugin* 470 nm light effectively killed *P. aruginosa* at all dose levels, but only killed *S. aureus* at 10 and 15 J cm^{-2}
Al-Watban *et al* [40]	442 488 + 514.5 632.8 780 830	SD rat	20 (all) 19 (Ar)	All lasers were better than control but He–Ne was most effective and He–Cd was least effective, three times weekly
Adamskaya *et al* [43]	470 (LED) 630 (LED)	Rat	30	470 nm light significantly influences wound healing
Al-Watban *et al* [42]	(488–514) Ar; (670) krypton	SD rat	20 80 100 140	20 J cm^{-2} Ar laser (488 nm) was most effective in wound healing; 140 J cm^{-2} Kr was inhibitive
Poon *et al* [44]	532 Q-switch; Nd : YAG	Human fibroblast	0.8	Significant delay in collagen remodeling activity and increase in SCF and b-FGF content

Al-Watban *et al* [40, 41] have compared the wound treatment by using various light sources including 442, 488, 632.8, 780 and 830 nm by fixing the energy density at 20 J cm^{-2} and treatment schedule. Most of the results have revealed stimulation in wound healing as compared to the control. He–Ne with dose of 20 J cm^{-2} has been attributed the most effective treatment, whereas the He–Cd violet–blue laser was the least. This meant the red laser was better for treatment than the violet–blue laser. The Al-Watban group [42] has conducted a similar investigation by using argon blue–green laser (488–514 nm) and krypton red laser (670 nm). They have found that the argon laser with dose 20 J cm^{-2} has shown more effective treatment than the krypton laser. Furthermore, zero biostimulation at dose of 80–100 J cm^{-2} was realized but inhibition occurs at 140 J cm^{-2}. Consequently, the blue–green laser is more appropriate for wound treatment than the red laser. This is contradictory with previous results [40]. Adamskaya *et al* [43] have conducted wound treatment by combining two light emitting diodes (LED). The LED was comprised of blue light (470 nm) and red light (630 nm) that was used to treat an excision rat model. Significantly, blue light contracted the wound size better than and enhanced epithelization compared to red light.

Poon *et al* [44] have accomplished *in vitro* biostimulation of dermal fibroblasts by using 532 nm Q-switched Nd : YAG. The laser was operated at a maximum energy of 200 mJ per pulse with pulse duration of 4 ns, in various modes including single shot and repetitive at the rates of 1, 2, 5 and 10 Hz. There is no significant difference in collagen synthesis. But the delay in collagen remodeling activity between the stimulated fibroblasts and controls is noticeable. Therefore, wound healing may be delayed without typical clinical features of infection [45]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the common aerobes found in the human tract and on the skin. They commonly cause skin infections and damage to tissues. A blue laser is usually used to kill bacteria *in vitro* such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. LED light at lines 405 and 470 nm is effective on bacteria such as *Staphylococcus* and *Pseudomonas aeruginosa* [46]. The LED of 405 nm is capable of destroying *Pseudomonas aeruginosa* and *Staphylococcus aureus* achieving a killing rate of 95 and 90%, respectively. All dose levels of 470 nm light are capable of destroying *Pseudomonas aeruginosa*, whereas bacteria *Staphylococcus aureus* can only be destroyed at 10 and 15 J cm^{-2}. Thus, blue light can produce a bactericidal effect.

4.2. Visible red laser

In this section, 40 cases have been studied. The range of lasers used in this wound treatment are listed in table 2. Almost half of them exploit lasers at 632.8 nm; this is the most popular wavelength used to expose wounds. Kana *et al* [47] have applied low and high energy density lasers for the wound treatment. They used energy densities of 4 and 20 J cm^{-2} and daily exposure on rat wounds with 632.8 nm. The low irradiation energy density showed a faster contracted wound area than the control group. This indicates that the energy density of 4 J cm^{-2} is capable of accelerating wound healing. In contrast, the high dose irradiation has no effect on the healing process. This finding is matched by Mester's result [7]. Other related work is also reported by Surinchak *et al* [48] and Lyons *et al* [49]. Both groups have claimed that LLLT is capable of breaking the strength of rat skin following irradiation with energy not exceeding 4.5 J cm^{-2}.

The types of samples also influence the outcome of wound treatment. No significant effects are realized when rabbit, swine and horse were used as samples [48, 50–52]. Ghamsari *et al* [53] have studied the effect of He–Ne on suture wounds of the teat in dairy cattle. The collagen fibers and tensile strength changed quite significantly after being treated by He–Ne radiation. Different species of animals may have different skin components, hence the characteristics of penetration depth of cellular molecules also differ. Some studies indicate that linearly polarized light can survive to propagate through long distances in biological tissue [54]. Al-Watban *et al* [41] utilized linear polarized He–Ne to cure an artificial wound with energy density in the range up to 60 J cm^{-2}. The acceleration of 27% in healing time and 49% in contraction area of wound was achieved after administering a dose of 25 J cm^{-2}. Al-Watban *et al* [40–42, 55–60] claimed that acceleration in wound healing is dependent upon the dose. The rate of healing process will increase up to a certain stage. The stimulatory effect was observed to decrease and inhibit beyond the optimum level. Photobiostimulation effect was dependent on the energy density of either *in vivo* or *in vitro* target. *In vitro* cellular studies [61–64] using human skin fibroblasts, and He–Ne lasers at various energy densities were utilized. The results were sufficient to produce measurable changes causing an increase in procollagen production. Although these findings indicate that the human skin fibroblast had displayed optimum effect at dose 5 J cm^{-2}, no stimulatory effect is revealed at a dose lower than 0.5 J cm^{-2}. Meanwhile, cellular damage occurs after the given dose is exceeded by 10 J cm^{-2}. High doses are usually used in photodynamic therapy [10].

Photobiostimulation is not only dose-dependent, but also wavelength-dependent. In the 20th century, most research was conducted using wavelengths 635 , 636 and 660 nm. The results showed a lack of stimulatory action either in low or high dosages [65–68]. The 670 nm laser was developed in the 20th century. The stimulatory function is understood to be different compared to a wavelength in the range 635–660 nm. An opposite finding is noted, whereby the healing process becomes more effective at a lower dosage.

Puglzese *et al* [69] have exploited GaAlAs laser at 670 nm with an output power of 9 mW. A Wistar rat was used as target. Laser radiation with low energy showed a stimulating effect on the target. An inhibiting effect is obtained after exposure to high-energy radiation. Referring to table 2, 4 J cm^{-2} is the dominant dosage to enhance the healing process, with the results in good agreement with several other researchers [69–71].

4.3. Infrared laser

Infrared radiation is divided into three categories: near-infrared (0.8–1.5 μm), middle-infrared (1.5–5.6 μm) and far-infrared radiation (5.6–10 000 μm). Near, middle and far-infrared rays have different photobiological effects [72]. Near-infrared wavelengths are weakly absorbed and penetrate deeply into the tissue (this penetration is, however, limited by optical scattering). In the middle- and far-infrared, water absorbs intensely, with light then only having very superficial effects [73]. The schematic diagram in figure 2 illustrates the effect of an infrared beam on tissue.

As the laser penetrates and is absorbed by soft tissue, a stimulatory effect occurs due to the activation of adenosine triphosphate (ATP) through bio-reaction. Table 3 summarizes the studies that involve infrared laser to stimulate the target. Grossman *et al* [74] conducted treatment by using a near-infrared diode laser at 780 nm with an output power of 6.5 mW. Proliferation of a culture of normal human keratinocytes *in vitro* was studied at various energy densities from 0 to 3.6 J cm^{-2}. Proliferation *in vivo* condition was also conducted in order to accelerate wound healing. The 780 nm-irradiation was claimed to induce a positive effect on wound healing.

LLLT has shown a variety of effects including increased maturation of collagen, fibroblasts and capillary vessels. The treatment was also capable of reducing pain and decreasing inflammation [75–79]. Laser therapy has been carried out using near-infrared at 830 nm with an output power of 10–40 mW. The healing process has shown a stimulation effect when treated with low energy density not exceeding 5 J cm^{-2} but tends to inhibit as the doses approach 20 J cm^{-2} [77, 80–82].

The healing process was also studied by comparing LLLT treatment with other method. As an example, comparisons between laser and ultrasound have been reviewed. A GaAs laser at 830 nm with an output power of 30 mW was utilized. The sample was exposed daily to a laser for interations of

Table 2. Wound healing studies involving red laser wavelength.

Authors	Target	λ (nm)	Energy density, (J cm^{-2})	Power (mW) (*mW cm^{-2})	Schedule	Outcome
Kana [47]	Rat	632.8	4	—	Daily	Increased collagen synthesis
			20			Inhibit
Surinchak [48]	Rabbit	632.8	1.1	—	Every third day	No significant effect
			2.2			
Surinchak [48]	Rat	632.8	2.2	—	Twice daily	Increased 55% breaking strength
			4.5			No significant effect
Hunter [50]	62 swine	632.8	—	64*	—	No significant effect
Lyons [49]	Rat	632.8	1.22	1.56	Every other days	Enhanced healing
Frets [51]	8 horse	632.8	45.9	13	—	No significant effects
Al-Watban [41]	SD rat	632.8	20	10.5*	6 times weekly	Accelerated healing
			25			Optimum dose in healing
			60			Inhibit healing
Atabey [52]	38 rabbits	632.8	3.8	5	15 min daily	No significant wound contraction but epidermal thickening, increased fibroblast and dermal vascularity
Al-Watban [40]	SD rat	632.8	20	—	3 times weekly	Enhanced healing
Ghamsari [53]	16 dairy cattle	632.8	3.64	8.5	30 s for 10 d	Enhanced healing
Nunes [100]	15 rat	632.8	1	10	Daily for 3 d	No significant effect
Hawkin [10]	Human skin fibroblast	632.8	0.5	—	Single dose	Increased fibroblast
			2.5			Increased fibroblast
			5			Increased fibroblast
			10			Cellular damage
Hawkin [61]	Human skin fibroblast	632.8	0.5	3*	2 d	No significant effect
			2.5			Increase in chemotaxis–chemokinesis and haptotaxis
			5			Optimum dose, enhanced healing
			10			Worse, DNA damage
			16			Worse, DNA damage
Rabelo [101]	50 rat	632.8	10	15	17 s	Enhanced healing, less intense inflammatory
Yasukawa [102]	SD rat	632.8	—	8.5	Every other day	Better than control
				17.0		Optimum dose, enhanced healing
Houreld [62]	Human skin fibroblast	632.8	5	—	Day 1 and day 4	Increased migration cell, no DNA damage and no cytotoxicity
			16			Inhibit and damage
Evans [63]	Human skin fibroblast	632.8	5	18.8	Daily	Enhanced healing
			16			No significant effect
Houreld [64]	Human skin fibroblast	632.8	5	—	—	Improved wound healing
Hedge [103]	105 rat	632.8	1	7	Single irradiance	Lowest effect
			2			Low effect
			3			Optimum dose
			4			Similar with optimum effect
			5			Low effect in laser group
Nussbanm [65]	70 SD rat	635	1	—	3 times weekly	No significant effects
			20			Worse
Sekhejane [66]	Human skin fibroblast	636	5	95	Single dose	Enhanced healing
Walker [67]	36 mice	660	0.5	15	3 times weekly	No significant effect
			1.5			No significant effect
			4			No significant effect
Gonzaga [68]	24 rat	660	20	—	7 d	Facilitates myofibroblast and proliferation
Al-Watban [42]	SD rat	670	140	—	—	Worse
Medrado [70]	72 Wistar rat	670	4	9	Single dose	Enhanced healing
			8			No significant effect
Puglzese [69]	72 Wistar rat	670	4	9	Single dose	Enhanced healing
			8			No significant effect
Do Nascimento [104]	18 Wistar rat	670	—	2	7 d	Enhanced healing
				15		Enhanced healing
				25		Optimum dose
Gal [105]	49 rat	670	30	—	Daily	Enhanced healing
Medrado [106]	112 Wistar rat	670	1	9	Daily	Enhanced healing

(*Continued*)

Table 2. (Continued)

Authors	Target	λ (nm)	Energy density, (J cm^{-2})	Power (mW) (*mW cm^{-2})	Schedule	Outcome
Reis [71]	32 rat	670	4	9	—	Enhanced healing
de Oliveira Guirro [107]	50 Wistar rat	670	4	—	—	No significant effect
			7			No significant effect
Pinheiro [93]	30 Wistar rat	685	20	—	Every other day for 7 d	Enhanced healing
			40			Worse
Rodrigo [80]	36 Wistar rat	685	20	30	Single dose	Worse

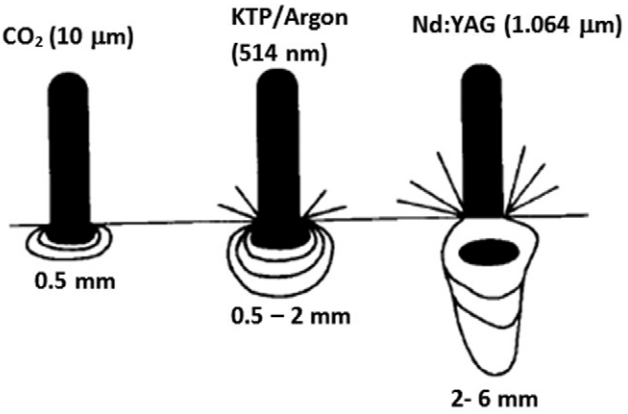

Figure 2. Laser tissue interaction—various wavelengths will reach different depths into tissues.

1 min or an energy density of 0.5 J cm^{-2}. Meanwhile, treatment with ultrasound (2 ms on; 8 ms off) was performed by exposing the sample using a power density of 0.1 W cm^{-2} for a duration of 5 min daily. Laser and ultrasound treatment have shown significant effects as compared to control group. Nevertheless, the ultrasound group did not achieve a statistically significant effect on wound healing [77].

GaAs laser at 904 nm is a popular light source in the infrared region. A continuous 904 nm GaAs was employed as a source for treatment. The energy density of 1 J cm^{-2} was exposed for 10 min per day. The treatment was carried out for 10 d. The finding was an increase in fibroblasts and wound breaking, i.e. it was beneficial for proliferation, as decreasing the macrophage and PNL means decreasing the inflammatory duration [79].

GaAlAs diode laser at 980 nm is also used for stimulating wound healing processes. In this study, the laser was operated at variable power within the range 1.5–10 W. With such a long wavelength and high power laser, the beneficial effect can only be achieved after a short interaction time per treatment for long interval times [83, 84]. Table 4 lists the variation of powers and days of treatment using 980 nm. The interaction time for every treatment is kept constant at 1 s.

Histology was analysed including epitheliazation, cellular content, granulation tissue, collagen deposition and vascularity by microscopic observation. Treatment with 5 W for 2 d was found to significantly enhance the healing process [84]. LLLT will regenerate the lymphatic system during the process of healing, which affects the occurrence of oedema and adhesion. The quicker the fluid waste products oedema can be drained, the better the potential of wound healing. The lymphatic system is primarily responsible for the evacuation of this oedema. If the lymphatic system is destroyed by the incision, then the regeneration process of these lymph vessels will determine the evolution of the scar [85].

A number of studies have reported that longer wavelengths (in the far-infrared region) are also able to motivate the bioreaction to accelerate the healing process [86–88]. Nd : YAG laser in pulse mode, for example, has been employed in the wound healing process. Two pulse modes have been conducted: 20 pulses per second (pps) with power of 1.75 W and 30 pps with power of 3 W, respectively. Differences in the distribution of matrix proteins during healing and the coagulation of the tissues were revealed after exposure to low energy laser treatment. This explained the minimal scarring, contraction and pigmentation of the lasered tissues as compared to conventional incisions [89]. The success of Nd : YAG laser pulse for stimulating the matrix process during healing does not mean that this will also apply for the continuous mode.

Ribeiro *et al* [86] used human skin fibroblast cells and irradiated with variable lasers including 632.8, 830 and 1064 nm at low energy density level. This study showed that cells had a high degree of haptotaxis and migration as well as ATP luminescence at 632.8 nm but no response at 1064 nm. This in contrast with previous claims that far-infrared is also capable of accelerating the healing process. In order to ensure this claim, another longer wavelength laser was investigated. In this case, de Freitas *et al* [90] used CO_2 gas laser to quantify statistically the myofibroblasts and compared with conventional treatments in the rat model. The result confirmed that the conventional treatment showed an increase in the number of myofibroblasts during the healing which is far better than CO_2 laser therapy. The threshold energy density and intensity are biologically independent parameters. The independence is of practical importance, at least for medical applications. Clearly, photobiological effects are more dominant at a low energy density level based on the account of the success and the failure in most of the cold laser uses since Mester's pioneering work [91, 92].

4.4. Alternatives to laser light source

Research in this domain mostly covers low-level laser studies; however, due to the high cost and safety aspect there is a need to consider other alternative light sources [76]. Recently, LED

Table 3. Wound-healing studies involve infrared laser.

Author	Target	λ (nm)	Energy density	Power (mW) (*mW cm^{-2})	Schedule	Outcome
Al-Watban [40]	SD rat	780	20	—	3 times weekly	Improved healing
Grossman [74]	Culture normal human keratinocytes	780	0–3.6	6.5	Single dose	Enhanced proliferation
Arcangelo [108]	24 Wistar rat (hard palate)	808	—	4000	—	Worse
				6000		Worse
Gungormus [109]	18 Wistar rat	808	10	4000	—	Improved healing
Souil [110]	Rat	815	—	1500 (pulse)	—	Improved healing
Petersen [111]	6 horse	830	2	—	Daily	No effects
Vinck [76]	Fibroblast (old chicken embryos)	830	1	40	3 d	Improved healing (*no different with LED group)
Mendez [75]	60 Wistar rat	830	—	—	—	Increased maturation collagen but no reduced cell inflammation
Lanzafame [82]	Rat	830	5	—	—	Improved healing
Rezende [81]	48 rat	830	1.3	60	Single dose	Optimum dose for improved healing
			3.0			No effects
Rodrigo [80]	36 Wistar rat	830	20	50	Single dose	Worse
Tikiz [77]	32 Wistar rat	830	0.5	30	—	Improved healing
Lowe [112]	50 mice	890	0.18		3 times weekly	No effects
			0.54		Pulse 270 Hz	No effects
			1.45			Optimum dose for improved healing
Ezzati [113]	67 rat (burn)	890	2.3		Pulse 3000 Hz	No effects
			11			Improved healing
Longo [114]	16 rat	904	3	3000 Hz	5 d	Improved healing
			3	1500 Hz		No effects
Skinner [115]	Fibroblast procollagen production	904	0.01–0.5	—	1–4 d	Optimum dose for healing
Pereira [116]	NIH 3T3	904	1 (6 h interval)	—	6 d	Optimum improved healing
			2 (6 h interval)			No effects
			2 (6 h interval)			No effects
Demir [79]	124 mice	904	1	6	10 d	Improved healing
Herascu [117]	Patient	904	—	—	—	Improved healing
Silveiro [118]	Rat	904	3	15–30	Daily for 10 d	Improved healing
Silveiro [119]	30 Wistar rat	904	5	15–30	After trauma 2, 12, 24, 48, 72, 96, 120 h	Improved healing
Sanati [120]	30 rat	904	2	*20.6	Every other day	Improved healing
		632.8	2	*31.7		No effects
Kawalec [84]	72 mice	980	18	5000	Every other day	No effects
					Every 4 d	Optimum dose
Kawalec [84]	72non diabetic mice	980	36	10,000	Every other day	No effects
					Every 4 d	No effects
Skopin [83]	Fetal human skin fibroblast	980	—	1500–7500 *73	Single dose (2 min)	Improved healing
Skopin [83]	Fetal human skin fibroblast	980	—	4500	Single dose 50 s	Improved healing
				*73	Single dose 2 min	Improved healing
					Single dose 15 min	No effects
Skopin [83]	Fetal human skin fibroblast	980	—	1500–7500	Single dose (2 min)	Improved healing
				*97		No effects
				*120		
Romanos [89]	Rat	1064		1750	Single dose	Improved healing
				3000		
Ribeiro [86]	Rat	1047	1	—	—	Improved healing with parallel polarized
Houreld [64]	Human skin fibroblast	632 830 1064	5			632.8 nm beneficial and 1064 nm worse in healing
Yu [88]	60SD rat	1000–12 000			Single dose, 30 min, 45 min, 60 min	Optimum duration 45 min for healing
Lanbach [87]	12 volunteers inner forearm	1500			Single irradiation	Epidermis recovers fast

Table 4. Treatment with GaAlAs 980 nm for treatment time of 1 s.

Power (W)	Days of treatment	Energy density (J cm^{-2})
5	2	18
5	4	18
10	2	36
10	4	36

has been favoured as an alternative source for light-based therapy in medical applications. Several researchers tried to improve the healing process by non-coherent light at different wavelengths. Table 5 lists the related works that deal with non-coherent light sources. Early studies by Pinheiro *et al* [93] irradiated four equidistant points with laser light (685 nm) or illuminated with wide range polarized light (400–2000 nm), both with doses of 20–40 J cm^{-2}. Wounds treated by laser therapy with a dose of 20 J cm^{-2} showed mild hyperemia, inflammation varied from moderate to intense, larger number of myofibroblasts without re-epithelialization. By increasing the dose to 40 J cm^{-2}, exuberant neovascularization, severe hyperemia, moderate to severe inflammation, large collagen deposition and fewer myofibroblasts were observed. As a result, 685 nm laser therapy is capable of increasing collagen deposition and better organization on healing wounds. The number of myofibroblast was increased by using polarized light with low dosage exposure.

Blue LED has great potential as light therapy for wound healing. It can significantly influence biological systems, improving perfusion by releasing nitric oxide from nitrosyl complexes with hemoglobin in a skin flap model in rats. A comparison between red LED (630 nm) and blue LED (470 nm) has been reported by Adamskaya *et al* [43]. Although blue light does not penetrate tissue as deep as red light, blue light significantly contracts wound area and decreases keratin-1 mRNA based on planimeter measurement and histology analysis.

In more complicated matters, debate has occurred regarding determining whether the coherent and monochromatic laser is a better performer than non-coherent light such as LED or a filtered lamp. Comparisons have been made which prove that LED has yielded a more beneficial stimulation effect than LLLT [76, 94]. The results have shown that the effects of a serial LED probe such as green (570 nm) and red (660 nm) were found significantly higher than a low-level light probe. Infrared LED and LLL source provided a higher number of cells than the control cultures but no significant statistical difference. According to the amount of proliferation, the green probe yielded a significantly higher number of cells than red, infrared and low-level laser. Other related work is also reported by Demidova-Rice *et al* [94]. A comparison study has been conducted between four different wavelengths in the ranges of red and near-infrared light centered at 635, 670, 720, and 820 nm and a coherent beam of 633 nm. An 830 nm light source has revealed the most pronounced results in stimulating wound healing. However, no significant difference is observed between non-coherent 635 nm and coherent 633 nm in stimulation action in the rat model.

4.5. Combination wavelength

Previous studies mostly performed with a single light source. However, there are some findings indicating the positive effect in healing processes by combining two or more energy sources. The combination of different energy sources can comprise laser, ultrasound, ultraviolet, electric current, magnetic field and microwave. Related works dealing with combination sources are summarized in table 6. Papageorgiou *et al* [95] have carried out a study on acne vulgarism treatment. A comparison treatment was conducted by using blue light (415 nm), mixed blue and red light (415–660 nm), cool white light and 5% benzoyl peroxide cream. The results showed that the laser treatment with mixed blue and red light was effective in inflammatory lesions. Guffey *et al* [96] exploited blue laser 405 nm combined with infrared laser 880 nm on *staphylococcus aureus* and *pseudomonas aeruginosa* to depress and reduce the number of bacteria colonies. They claimed that such a combination has shown the most effective way to kill both bacteria.

Generally, blue light is commonly used for bactericide. Combination red and infrared light has been reported by Braverman *et al* [97], who dealt with 72 rabbits. Helium–neon laser radiation (He–Ne; 632.8 nm) and pulsed infrared laser radiation (IR; 904 nm) were combined to irradiate skin wounds. The tensile strength for the laser treated groups was more significant than the non-irradiated group; however, there were no significant differences in statistical data between the laser group for wound healing, collagen area and epidermal growth. Similar work was also reported by Lievens [85], who studied regeneration of the lymphatic system during the process of wound healing by combining cw He–Ne 632 nm and pulse infrared laser 904 nm. The frequency of the pulsed infrared laser is 1000 Hz with an energy density of 2.1 J cm^{-2}. The energy density of He–Ne laser is 1.2 J cm^{-2} to treat 500 mice. The treatment is carried out twice daily. Laser treatment has enhanced the adhesion, oedema and the lymph vessels, thus accelerating the vein regeneration process of blood and lymph vessels during wound healing. Simunovic *et al* [122] studied this using a human specimen. They claimed that the wound healing, pain relief and functional recovery of patients was significantly improved for the group of patients treated by LLLT compared to untreated patients.

The combination of 685 and 830 nm reveals increased collagen production and organization [7]. Better repair of wounds was found by using energy density of 20 J cm^{-2} as compared to that with 50 J cm^{-2}. Combining the light exposure is more effective than using a single laser. In contrast, a similar experiment [80] obtained different results. Histological analysis was used to investigate the systemic action and repair process of wounds produced on the backs of rats and treated with red, infrared or both lasers applied directly or indirectly to the wounds. The combined application of red and infrared lasers resulted in the most evident systemic effect on the repair of skin wounds produced in rats.

Recently wounds have been treated by combining two light sources comprised of 685 and 830 nm [98]. Lack of beneficial effect is revealed after irradiation with high energy density of 22 J cm^{-2}.

Table 5. Wound healing that involves non-coherent light sources.

Author	Target	Wavelength (nm)	Energy density (J cm^{-2})	Power (mW)	Schedule	Outcome
Vinck [121]	Embryonic chicken fibroblast	570	0.1	10	3 d	Improved healing
Vinck [76]	Fibroblast (old chicken embryos)	Laser 830 LED 570 660 950	 1 0.1 0.53 0.53	 40 10 80 160	3 d	Improved healing (but no difference with 830 nm laser)
Demidova-Rice [94]	139 mice	Laser 632.8 LED 635 670 720 820	1	—	—	820 nm optimum wavelength and 635 nm second advanced, no difference between 632.8 nm and 633 nm
Pinheiro [93]	30 Wistar rat	400–2000	20	—	Every other days for 7 d	Improved healing (more benefit than 685 nm)
Al-Watban [57]	893 SD rat	Laser 532 633 810 980 10 600 LED 510–872	4.71	—	Three times per week	633 nm improved healing
Adamskaya [43]	Rat	470 630 (LED)	0.5 J cm^{-2}	1000	For 5 d	Blue light significantly influences wound healing

Table 6. Wound healing involving combination wavelengths.

Author	Target	Wavelength (nm)		Energy density (J cm^{-2})	Power, mW		Schedule	Outcome	Remark
Papageorgiou [95]	107 patients	415 ± 20	660 ± 20	—	4.23	2.67	Daily for 12 weeks	Optimum dose	Better than single LED
Lievens [85]	500 mice	632	904	(1.2 + 2.1)	5	6800	Twice daily	Improved healing	Compared to control
Simunovic [122]	74 patients	632.8	904 (pulse)	—	—	—	—	Improved healing	Compared to control
Braverman [97]	72 rabbits	632.8	904 (pulse)	(1.65 + 8.25)	—	—	Daily for 21 d	No effect	No significant difference with single laser
Noudeh [98]	20 rats	670	810	(10 + 12)	500	250	—	No effect	Compared to control
Rodrigo [80]	36 Wistar rats	685	830	20	30	50	Single	Worse	Most evident systemic effect on healing
Mendez [75]	60 rats	685	830	20	35	35	—	Optimum dose improved healing	Combination better than single laser
				50				No effect	
Nussbaum [99]	20 patients	820	30 super-luminous diode	—	15	—	Three times weekly	Worse	Compared with US/UVC

Combinations were also organized between light source and other techniques. For example, two light source treatments were compared between ultrasound and ultraviolet-C (US/UVC) [99]. The light group combined a beam of 820 nm (energy density of 120 J cm^{-2}) with non-coherent light of 4 J cm^{-2} operating in pulse mode with a repetition rate of 5000 pulses per second (pps). In this study, humans were used as targets. The patients were exposed three times weekly with the combination of light sources; and five times weekly for the combination of ultrasound (US) group (3 MHz) with

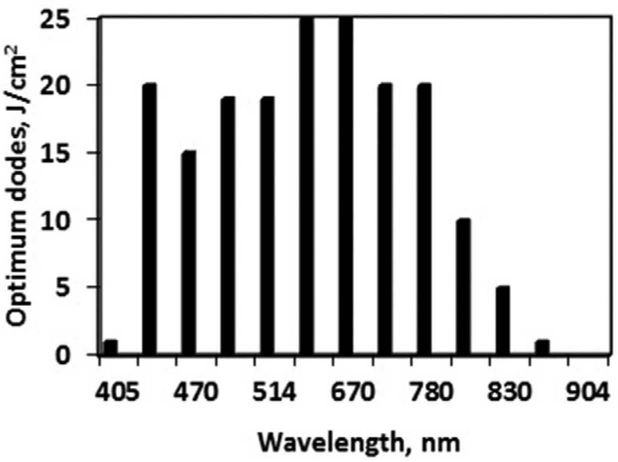

Figure 3. Dependence of optimum dose on wavelength.

ultraviolet-C group (250 nm). The combination of US/UVC group has shown an advantage in comparison to the light sources group. This is possibly due to the tissues being absorbent without selection of the optimum characteristic [99].

Overall, the combination of irradiation was found to be more effective in comparison with single irradiation. The longer wavelength indicates a significant difference and enhances the effective healing.

5. Summary and future trend

Optical technology is a promising technique to replace conventional wound treatment. Less cream or any medicine applied on the wound is normal practice in a conventional method. Lasers and non-coherent light sources like LED can surpass traditional medicine, resulting in less pain, faster and simple treatment. Laser wound treatment is a non-destructive technique, which is non-touch, and directly illuminated on to the wound. As a result, the wound area is sterilized, killing the bacteria and enhancing the collagen production (this is an important agent in the healing process). No need to clean, apply medicine or bandages. Such treatment is very promising, economical and fast. From the many light sources that have been discussed earlier, blue and near-infrared lasers have shown better performance in wound healing process. Therefore, further investigation using these two light sources is needed to as well as maybe the potential to combine both of them for better treatment in wound healing. In general, the relationship between the optimum doses with respect to wavelength is shown in figure 3. There is an optimum dose that is at 25 J cm^{-2} corresponding to a wavelength in the red region. The blue and near-infrared have the smallest optimum dose of about 1 J cm^{-2} for wound healing treatment.

6. Conclusion

In summary, low-level laser therapy at the appropriate dosimetric parameter can provide an acceleration effect in wound healing. The bio-stimulatory effects were dependent on the energy density or doses and laser wavelengths. Different light sources had different interactions with wounds. The visible laser region is capable of accelerating the wound repair via enhanced proliferation of cells and a reduction in inflammation. With *in vivo* sampling, the appropriate dose appears to be in the range 1–5 J cm^{-2}, corresponding to a wavelength of 632.8 nm. It becomes more effective when administered on a daily basis. *In vitro* sampling, the suitable dose to stimulate human fibroblast is in the range 0.5–5.0 J cm^{-2}. For an infrared laser, the frequency of administering therapy should be less than visible laser therapy and the energy dose around 0.5–10.0 J cm^{-2}. There is still a lack of attention given to energy doses higher than 10 J cm^{-2}. The infrared laser will achieve optimum treatment with high power dose and short exposure time. Apparently, a combination technique may join the effects of antibacterial and anti-inflammatory action in order to accelerate the healing process. Larger differences between two wavelengths perhaps increases the stimulatory effects. Polarized light would be more effective than an unpolarized light source. Finally, it is better to highlight that there is no significant difference between laser and LED effects on wound healing processes.

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