Sudan University of Science and Technology
Collage of Graduate Studies

Effectiveness of Low-Level Laser Therapy in Pain Reduction and Healing Acceleration after Undisturbed Tooth Extraction

فعالية العلاج بالليزر منخفض المستوى في تقليل الألم وتسريع الشفاء بعد قلع الأسنان غير المضطرب

A Dissertation of Graduation Project as Partial Fulfillment for the Requirements of Degree of Postgraduate Diploma in Laser Applications in Medicine (Dentistry)

By

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DEDICATION

To my family,

Whose their love and encouragement made me able to have such success and honor.

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Abstract

Tooth extraction considered to be a commonly performed procedure in the developing countries, in which it has a great effect of general health, quality of life and socioeconomic status. Despite of being preventable, there are many causes of tooth extraction including caries, periodontal disease, failed endodontic treatment and fractures. This associated with some complications such as pain, bleeding, failure of socket healing. Further, in extreme rare cases tooth extraction can lead to osteomyelitis of the jaw bone.

Laser therapy has a long history of medical uses and has shown promise in dental treatments. Low-level laser therapy (LLLT) is a significant branch of medicine where low-power lasers are used on living tissues to stimulate and improve cell function or relieve pain. Several studies have confirmed the reduction of pain, swelling, improvement of revascularization and wound healing. Several studies have shown that after surgical removal of the lower third of the molars, laser treatment had a beneficial effect on reducing pain, edema and trismus.

This study aims to evaluate the use of two different wavelengths of low-level laser in reducing pain and bleeding after tooth extraction. Thirty patients participated in the study in which 15 patients treated by a 980 nm diode low-level laser, and the other 15 patients treated by 650 nm diode low-level laser.

The results of the present study showed that the diode low-level laser with a wavelength of 980 nm was significantly reduced pain and bleeding in comparison with diode laser 650 nm.

المستخلص

يعتبر قلع الأسنان إجراءً شائعًا في البلدان النامية، وله تأثير كبير على الصحة العامة ونوعية الحياة والحالة الاجتماعية والاقتصادية. على الرغم من إمكانية الوقاية منه ، إلا أن هناك العديد من أسباب قلع الأسنان بما في ذلك: تسوس الأسنان، وأمراض اللثة، وفشل العلاج اللبي، والكسور. أحد مضاعفات خلع السن هو الألم والنزيف وفشل التئام السنخ ، وفي حالات نادرة للغاية يمكن أن يؤدي إلى التهاب في عظم الفك.

العلاج بالليزر له تاريخ طويل من الاستخدامات الطبية وقد أظهر نتائج واعدة في استخدامات الأسنان. العلاج بالليزر هو فرع مهم من فروع الطب يستخدم أشعة الليزر منخفضة الطاقة على الأنسجة الحية لتحفيز وتحسين وظيفة الخلايا أو تخفيف الألم. أكدت العديد من الدراسات تقليل الألم والتورم وتحسين إعادة تكوين الأوعية الدموية والتئام الجروح. أظهرت العديد من الدراسات أنه بعد الإزالة الجراحية لأضراس العقل السفلية، كان للعلاج بالليزر تأثير مفيد في تقليل الألم والوذمة وعدم القدرة على فتح الفم.

تهدف هذه الدراسة لتقييم استخدام طولين موجيين مختلفين من الليزر منخفض المستوى في تقليل الألم والنزيف بعد قلع الأسنان. شارك ثلاثون مريضًا في الدراسة التي كان فيها 15 مريضًا تم علاجهم بليزر منخفض المستوى ذو طول موجي 980 نانومتر ، بينما كان لدى 15 مريضًا ليزرًا منخفض المستوى ذو طول موجي 650 نانومتر.

أظهرت نتائج الدراسة الحالية أن ليزر الثنائي منخفض المستوى بطول موجي 980 نانومتر قد قلل بشكل ملحوظ من الألم والنزيف مقارنة بليزر 650 نانومتر

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Abbreviations

LLLT: Low-level laser therapy.

VAS: Visual analogue scale.

CHX: Chlorhexidine.

mW: Milli-watt.

nm: Nano-meter.

J: joule

UV: Ultra violet

CO_2 ; Carbon dioxide

Nd: YAG: Neodymium-doped yttrium aluminum garnet

Chapter one

1.1 Introduction

Tooth extraction remains a considerable commonly performed procedure in developing countries (Jafarian and Etebarian, 2013, Saheeb and Sede, 2013). Tooth loss has a compelling socioeconomic, quality of life, general health, and psychological sequela (Jafarian and Etebarian, 2013, Taiwo, 2014). Extraction has become a global public health concern of massive proportion (Alesia and Khalil, 2013, Taiwo, 2014). Despite being preventable, dental caries and periodontal disease remain the most common reasons for tooth extraction, especially developing nations(Dixit et al., 2010, Kashif et al., 2014). Other indications for tooth extraction include residual roots, tooth fracture, failed endodontic treatments with the persistence of periapical granulomas or cysts and third-molar impaction(Vettori et al., 2019).

After tooth extraction, numerous histochemical and histological responses occur to heal the extraction site. The hemorrhage in the alveolar socket is followed by the formation of a blood clot, the organization of a fibrin clot, the migration of the epithelial layer over the wound surface, the resorption of damaged tissues, and lastly the new bone formation (Amler et al., 1960).Complications after tooth extraction are scarce and mostly minor. Mild to moderate pain is experienced in some patients following tooth extraction(Bui et al., 2003).

Laser therapy has a long history of medical uses and has shown promise in dental uses. There is evidence that laser therapy is a useful addition to conventional routine dental treatment and is becoming increasingly popular with dentists (Elson and Foran, 2015).Low-level laser therapy (LLLT) is a new branch of medicine that uses low-power lasers on living tissues to stimulate and improve cell function or relieve pain. Several studies have confirmed the reduction of pain and swelling and improvement of revascularization and wound healing(Eshghpour et al., 2017, Farivar et al., 2014, Holanda et al., 2017, Moosavi et al., 2016, Pol et al., 2016, Sardari and Ahrari, 2016).There are conflicting reports about the effectiveness of laser treatments to reduce possible complications after tooth extraction. Several studies have shown that after surgical removal of the lower third of the molars, laser treatment had a beneficial effect on reducing pain, edema and trismus(Aras and Gungormus, 2009, Batinjan et al., 2014, Eshghpour et al., 2016, Kahraman et al., 2017, Kazancioglu et al., 2014, Landucci et al., 2016, Markovic and Todorovic, 2007, Merigo et al., 2015, Saber et al., 2012).However, some studies did not find any additional benefits of using low-level lasers over placebo in a variety of conditions, such as: pain relief after primary(Elbay et al., 2016), permanent(Paschoal and Santos-Pinto, 2012) teeth extraction, and reduction in pain and swelling after the third surgery(Amarillas-Escobar et al., 2010, Brignardello-Petersen et al., 2012, Lopez-Ramirez et al., 2012). The use of LLLT has shown anti-inflammatory effects due to its direct effect on lymphatic vessels (increased number) and blood vessels (decreased permeability). LLLT is a beneficial technique and can promote a variety of biological responses depending on the dose, wavelength, and the condition of the target area. These reactions include the acceleration of the tissue healing, improving bone repair, restoring normal nerve function after injury, moderating inflammatory reactions, stimulating analgesia, reducing swelling and regulating the immune system(Aras and Gungormus, 2010, Jovanović et al., 2004, Leung et al., 2012, Markovic and Todorovic, 2007, Roynesdal et al., 1993). LLLT improves protein absorption by activating macrophages and modifying intra-capillary hydrostatic pressure, thereby inducing the absorption of interstitial fluid with the consequent reduction in swelling (Lievens, 1991, Markovic and Todorovic, 2006, Ozen et al., 2006).

1.2 Problem statement

Tooth extraction is the final stage of severe diseases that affect the teeth. Complications following tooth extraction are rare and mostly minor. Some patients experience mild to moderate pain following tooth extraction. One of the complication of tooth extraction is the presence of an empty socket due to failure of healing, it is annoying for most patients as it may be surrounded by sharp bony edges and filled with food debris after eating and cause disturbing pain. Due to the great number of patients who undergo tooth extraction worldwide, the prevention or treatment of likely complications becomes important(Ahrari et al., 2020).

Low-level laser therapy (LLLT) is a novel branch of medicine that employs low-power lasers to living tissues in order to stimulate and enhance cell function or relieve pain. The effectiveness of LLLT in reducing pain and swelling, stimulating nerve function, enhancing revascularization and improving the wound healing process has been confirmed in several studies (Eshghpour et al., 2017, Farivar et al., 2014, Holanda et al., 2017, Moosavi et al., 2016, Pol et al., 2016, Sardari and Ahrari, 2016).

There for the aim of the present study is to assess the post-operative satisfaction and the effectiveness of Low-Level Laser Therapy (LLLT) in pain reduction and healing acceleration after undisturbed tooth extraction.

1.3 Objectives

1.3.1 General Objectives

To evaluate the effectiveness of LLLT in relieving pain after non-disturbed teeth extraction.

1.3.2 Specific Objectives

- To assess the degree of pain perception after extraction and application of LLLT using a 980 nm diode laser using the visual analogue scale (VAS).
- To assess the degree of pain perception after extraction and application of LLLT using a 650 nm diode laser using the visual analogue scale (VAS).
- To assess the difference in pain relief between the 980 nm and 650 nm wavelengths.
- To measure the post-operative satisfaction of patients after extraction and application of LLLT using the visual analogue scale (VAS).

Chapter Two
Literature Review

Tooth extraction is one of the last dental procedures that should be considered. Decreased number of teeth can lead to malnutrition and reduced quality of life (Aida et al., 2006). The number of tooth extractions can be used as an indicator of socioeconomic status and oral hygiene level(Chrysanthakopoulos, 2011). In modern dentistry, the process of alveolar socket healing after tooth extraction has become an important subject of research and discussion (Buser et al., 2004).

Tooth extraction was once described as a tissue amputation that may lead to functional, psychological, postural and local changes (Atwood, 1963). In fact, tooth extraction was initially considered to be a simple tooth loss, but local changes will occur, leading to changes in hard and soft tissues. The process of local changes in closing the wound and restoring tissue homeostasis is called "socket healing"(Araujo et al., 2015).

2.1Anatomic aspects of the tooth socket

The alveolar process may be defined as the bone tissue that surrounds a fully erupted tooth and it is formed in harmony with the development and eruption of the teeth. It is limited coronally by the bone margins of the socket walls, whilst an imaginary line that cuts the bottom of the socket in a perpendicular direction to the long axis of the root, limits it apically. Beyond such a line, the basal bone of the mandible or the maxilla can be found (Araujo et al., 2015).The morphological characteristics of the alveolar bone are related to the following aspects: (i) the size and shape of the teeth; (ii) the position of the teeth erupting; (iii) the inclination of the erupting teeth. In general, teeth tend to erupt and incline to a position outside the center of the basal bone (Pietrokovski and Massler, 1967).

2.2 Histological aspects of the tooth socket

The inner portion of socket walls is named “alveolar bone proper” or bundle bone (a histological term) and the remaining hard structure is called “alveolar bone”. The bundle bone is a lamellar bone, 0.2–0.4 mm wide, composed of circumferential lamellae, whilst the alveolar bone is also of the lamellar type but composed of concentric and interstitial lamellae and of marrow. In the bone bundle, the Sharpey's fibers are lined in such a way that they connect the periodontal ligament with the alveolar bone and the skeleton. On the contralateral side of the periodontal ligament, the tooth cementum coated with Sharpey's fibers connects the periodontal ligament with the dentin. Like root cementum and the periodontal ligament, the bundle bone is a tooth-dependent structure(Araujo et al., 2015).

2.3 Socket healing

Tooth extraction induces a series of complex and integrated local changes within the hard and soft tissues. These alterations arise in order to close the socket wound and to restore tissue homeostasis, and are referred to as "'socket healing" (Araujo et al., 2015), these changes include :

2.3.1 Dimensional changes

The dimensional changes occurring in the alveolar bridge after tooth extraction have been reported in several human studies(Atwood, 1963, Bergman and Carlsson, 1985, Johnson, 1963, Johnson, 1969, Pietrokovski and Massler, 1967, Pietrokovski et al., 2007, Schropp et al., 2003, Trombelli et al., 2008). After multiple tooth extractions and the use of complete removable prostheses, the alveolar ridge experiences a significant contraction in both the vertical and horizontal directions (Atwood, 1962, Atwood, 1963, Carlsson and Persson, 1967, Johnson, 1963, Johnson, 1969). Following numerous years of complete denture use, people may also go through an extensive variation in alveolar ridge reduction and a few may also showcase a completely resorbed alveolar ridge (Bergman and Carlsson, 1985). After a single tooth is extracted, the ridge shows a limited reduction in its vertical dimension, but the horizontal reduction is substantial. It is expected that: (i) there will be a reduction in the original width of the ridge by up to 50%; (ii) the extent of bone resorption is greater on the buccal side than on the lingual / palatal counterpart; and (iii) a large amount of reduction of the alveolar bone will take place in the molar regions(Araujo et al., 2015).It is clinically observed that the end of the socket healing process is due to the firm epithelial soft tissue closing the socket entrance and/or the radiological filling of the cavity with bone. It is expected that individuals will vary greatly in the time required to fully heal the socket(Carlsson and Persson, 1967, Johnson, 1963, Johnson, 1969, Schropp et al., 2003, Trombelli et al., 2008). The socket entrance may be restored between 10 and 20 weeks(Johnson, 1969, Tal, 1999) and radiographic bone fill observed between 3 and 6 months post-extraction (Schropp et al., 2003). While most dimensional changes involving the healing of socket occur in the first 3 months , the reorganization of the alveolar ridge can continue for up to a year after extraction (Brkovic et al., 2012, Schropp et al., 2003). It is reasonable to assume that the rate of socket healing is affected by biological differences between individuals, the size of the alveolar socket size (large versus small sockets), and the amount of surgical trauma induced during the extraction process (Araujo et al., 2015).

2.3.2 Histologic changes

The sequence of events that occur after tooth extraction has been described in both human and dog studies(Amler, 1969, Araújo et al., 2009, Araujo and Lindhe, 2005, Araujo et al., 2005, Araújo et al., 2006, Carlsson and Persson, 1967, Trombelli et al., 2008). In human studies, biopsies from the marginal element or from the central part of the healing sockets have been used to describe the healing events, while in animal studies (canine model), biopsies from the whole alveolar socket have been organized for histologic analyses. Although bone modeling and remodeling is 3 to 5 instances quicker in dogs than in humans(Araujo et al., 2015). The overall histological results of these studies showed a remarkable similarity between socket healing in dogs and humans. Therefore, it has been observed that the alveolar healing process can be divided into three consecutive and often overlapping phases: inflammatory; proliferative; and modeling / remodeling (Araujo et al., 2015).

2.3.3 Inflammatory phase

The inflammatory phase can be divided into two parts: blood clot formation and inflammatory cell migration. Immediately after tooth extraction, bleeding occurs and the socket filled with blood. The blood clot blocks the cut vessels and stops bleeding. Within 2-3 days, many inflammatory cells migrate into the wound to "clean" the area before new tissue can form. The combination of inflammatory cells, vascular sprouts and immature fibroblasts form the granulation tissue. When the site is sterilized, the granulation tissue is gradually replaced with a temporary matrix of connective tissue rich in collagen fibers and cells, and the proliferative phase of the wound healing process begins (Araujo et al., 2015).

2.3.4 Proliferative phase

The proliferative phase can also be divided into two parts – fibroplasia and woven bone formation and is characterized with the aid of using extreme and fast tissue formation. Fibroplasia includes the fast deposition of a The primary osteons can be once in a while strengthened with the aid of using parallel-fibered bone. Woven bone may be identified in the healing socket as early as 2 weeks after tooth extraction and stay sin the wound for several weeks. Woven bone is a provisional sort of bone without any load-bearing ability and therefore desires to get replaced with mature bone types (lamellar bone and bone marrow)(Araujo et al., 2015).

2.3.5 Bone modeling and remodeling phase

Bone modeling and remodeling is the third and final phase of the socket-healing process. Bone modeling is defined as a change in the shape and architecture of the bone, whereas bone remodeling is defined as a change without concomitant change in the shape and architecture of the bone. The replacement of woven bone with lamellar bone or bone marrow is bone remodeling, whereas the bone resorption that takes place on the socket walls leading to a dimensional alteration of the alveolar ridge is the result of bone modeling. Bone remodeling in humans may take several months and exhibits substantial variability among individuals (Carlsson and Persson, 1967, Trombelli et al., 2008). In a recent study, Lindhe al.(Lindhe et al., 2012) tested the tissue composition of biopsies from 36 people retrieved from previous socket sites with inside the posterior maxilla after >sixteen weeks of healing. The authors suggested that approximately 60–65% of the tissue volume was made of lamellar bone and bone marrow. Thus, the whole remodeling of the woven bone into lamellar bone and bone marrow may also take numerous months or years.

A few weeks after tooth removal, osteoclasts might be found around the crest of each buccal and lingual walls and on the outer and inner (bundle bone) parts of the socket. Bone modeling takes place equally on buccal and lingual walls, however as a result of the lingual bone is usually wider than the buccal bone wall, modeling results in bigger vertical bone loss at the skinny buccal plate than at the wide lingual wall. In addition, bone modeling takes place before bone remodeling, in such means that regarding simple fraction of the modeling process occurs within the initial three months of healing(Schropp et al., 2003).In summary, it can be said that the modeling and remodeling processes during the base healing lead to qualitative and quantitative changes in the edentulous area, which lead to a reduction in the size of the ridge (Araujo et al., 2015).

2.4 Stimulating factors

Initial wound healing responses are regulated by signaling molecules (i.e. growth factors and cytokines) such as: platelet derived growth factor, insulin-like growth factors, transforming growth factor beta and fibroblastic growth factors. They initiate cell migration, differentiation and proliferation as they interact with each other in highly ordered temporal and spatial sequences (Lalani et al., 2003). These growth factors act as angiogenic and mitogenic signals at the early stage of bone healing. Once activated, growth factors instigate a series of events via ligand–receptor interactions, including signal transduction, gene transcription, mRNA-directed protein biosynthesis and secretion of post-translational proteins(Hollinger and Wong, 1996). Fisher et al.(Fisher et al., 2004) evaluated the expression of growth factors during socket-healing events in a rabbit model. The authors observed that: (i) fibroblast growth factor-2 presented at higher levels at early time points, before returning to lower levels; (ii) vascular endothelial growth factor levels were maintained constant during healing; (iii) platelet-derived growth factor-A levels increased during the first days of socket healing; (iv) transforming growth factorbeta1 presented a small elevation at early time points; and (v) an increased expression of bone morphogenetic protein 2 was observed when osteoblast precursors accumulated and began to proliferate(Araujo et al., 2015).Trombelli et al. (Trombelli et al., 2008) studied modeling and remodeling of human extraction sockets and evaluated the expression of bone morphogenetic protein 7 during socket healing. The results demonstrated that bone morphogenetic protein 7 increased during early and intermediate healing phases, and a period of increased bone modeling and remodeling activity occurred, leading to the deposition of woven bone from provisional matrix. In summary, growth factors present multiple activities, generally with overlapping actions, and a simplistic characterization of their effects is not possible, or indeed appropriate.

2.5 Post extraction pain

According to Merriam Webster dictionary, pain as defined as : a localized or generalized unpleasant bodily sensation or complex of sensations that causes mild to severe physical discomfort and emotional distress and typically results from bodily disorder (such as injury or disease) (Webster, 2021).

One of the most common procedures in dental clinics and the most common task in oral and maxillofacial surgery clinics is the extraction of teeth (Rakhshan, 2015).Pain is also one of the most common post-operative complications of teeth extraction (Capuzzi et al., 1994, de Santana-Santos et al., 2013, Haraji and Rakhshan, 2015, Lago-Mendez et al., 2007, Pedersen, 1985) and can be caused by the release of pain mediators from injured tissues (El-Soud and El Shenawy, 2010, Haraji and Rakhshan, 2015). Pain is an important element in clinical practice (de Santana-Santos et al., 2013, Slade et al., 2004). and could even intimidate patients from seeking dental care(Bienstock et al., 2011, Haraji and Rakhshan, 2015, Wardle, 1984). It begins after the anesthesia subsides and reaches its climax levels during the first postoperative day(Haraji and Rakhshan, 2015, Haraji et al., 2013, Susarla et al., 2003). If dry socket or infection occur, the onset of inflammation will impede the alleviation of postoperative pain(Bloomer, 2000, Blum, 2002, Cardoso et al., 2010, Haraji and Rakhshan, 2015, Haraji et al., 2013, Kolokythas et al., 2010, Noroozi and Philbert, 2009, Penarrocha et al., 2001). In case of dry socket, the post-surgical pain will differ in pattern; it will intensify between the second and fourth post-surgical days(Bloomer, 2000, Blum, 2002, Cardoso et al., 2010, Caso et al., 2005, Haraji et al., 2013, Kolokythas et al., 2010, Noroozi and Philbert, 2009).

pain is considered a crucial part of practice (de Santana-Santos et al., 2013). Different measures have been suggested for this purpose, including systemic analgesics, irrigation with different agents and using analgesic/tranquilizer dressings(Bloomer, 2000, Blum, 2002, Cardoso et al., 2010, Haraji et al., 2013, Kolokythas et al., 2010, Larrazábal et al., 2010, Noroozi and Philbert, 2009). Also the application of chlorhexidine (CHX) gel might reduce post-extraction pain (Larrazábal et al., 2010).

2.6 Laser

The term LASER is an acronym for 'Light Amplification by the Stimulated Emission of Radiation' (Verma et al., 2012).laser is a form of electromagnetic energy that propagates in waves at constant speed. The basic unit of this radiant energy is called the photon or light particle. A photon wave can be defined by two basic properties. The first is the amplitude and the second is the wavelength. Amplitude gives the measurement of the amount of energy in the wave. The wavelength is the distance between two corresponding points on the wave.(Ballal et al., 2013).

2.6.1 Components of Laser System

The basic component of a laser is quite simple and includes a laser medium placed in an optical cavity, a pump power source and a cooling system. In order to contain and intensify the photon chain reaction, it is necessary to place this reaction within into an optical cavity (Ballal et al., 2013).

The optical cavity consists of two parallel mirrors located on each side of the laser or laser medium. The mirrors are separated by a fixed distance (d) to form a Fabry-Perot interferometer. The release medium can be a gas cylinder (CO_2, argon).), a liquid or solid crystal (garnet crystal, usually made of yttrium and aluminum, and added with chromium, neodymium, holmium or erbium). They can also be solid-state semiconductors made of metals such as gallium, aluminum, and arsenide. In this configuration, photons bounce off the mirror and re-enter the medium to stimulate the release of more photons. Collimating mirror; that is, photons that are completely perpendicular to the mirror reenter the active medium, while off-axis photons leave the laser process. Since the process is not completed at 100% and part of the energy is converted into heat, a cooling system is provided. If one mirror is totally reflective (M_2), the other mirror (M_1) is partially transparent. The light emitted through M_1 becomes a laser beam. The laser is named after the content of the active medium and their state of suspension. Optical feedback in the laser cavity The mirror system in the laser cavity can be imagined as a completely silver mirror (Ballal et al., 2013).

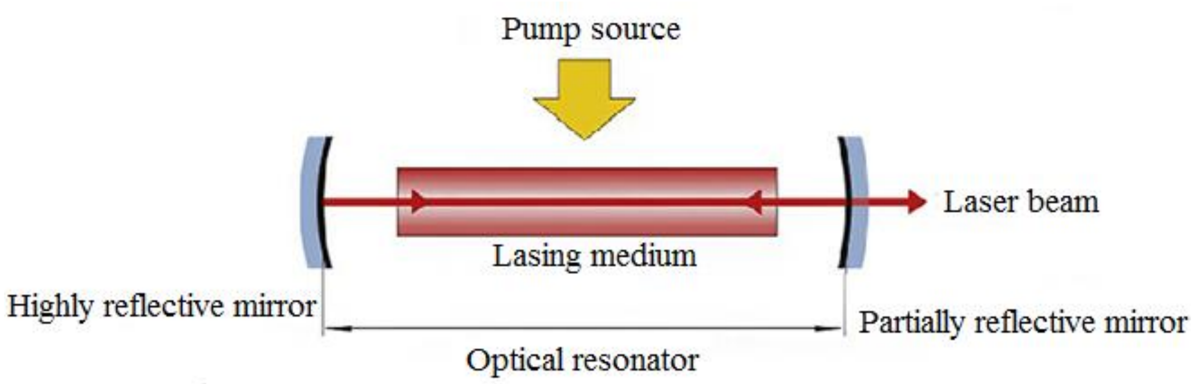

Figure 2.2 Components of laser device (Malíčková et al., 2018).

2.6.2 Properties of Laser Light

There are several remarkable properties of laser light that distinguish it from the normal light.

2.6.2.1 Monochromatism

Lasers emit light that is monochromatic or specifically has a single wavelength from UV to infrared. Lasers express one color. Lasers of different types emit a single wavelength or specific wavelengths and in fact they can be tuned to different wavelengths as the operator desires. This property is important for the high spectral power density of the laser beam(Ballal et al., 2013).

2.6.2.2 Collimation or (directionality)

The laser beam as it comes from the laser device has very little divergence. They do not diverge and run parallel to each other. The emitted beam has a constant size and shape. Most of the gas or solid-state lasers emits a laser beam with an angle of divergence of approximately one milli radian. This explains why laser light is extremely hazardous. Because the distance doesn't diverge, the laser light maintains brightness so it's still focused enough to be dangerous. But this property is important to good transmission through the delivery system(Ballal et al., 2013).

2.6.2.3 Coherence

The generated laser light waves are physically identical. They have the same amplitude and frequency. There are two types of laser light coherence, longitudinal and transverse. The type of longitudinal coherence represents the temporal coherence along the longitudinal beam, while the transverse or spectral coherence is referred to. Coherence refers to coherence across the beam. Coherence causes a laser beam to collimate over extremely long distances and enables the beam to be extremely finely focused. Any given laser beam can only be focused on a diameter that corresponds to the wavelength of the specific laser(Ballal et al., 2013).

Another property of laser light that sets it apart from other conventional light sources is its luminosity. This property arises from the parallelism or collimation of the laser light as it moves through space and maintains its concentration. Energy when the laser is focused on a small point. Doctors rely on the luminous focus of the laser beam to raise tissue temperature or to cut or vaporize tissue (Ballal et al., 2013).

2.6.2.4 Excellent concentration of energy

Lasers have excellent concentration of energy and hence it has got tissue targeting effect (Ballal et al., 2013).

2.7 Classification of Lasers

There are many classifications of laser based on wavelength, hazard, mode of operation and the most common classification is based on the active medium.

A) Based on active medium

- **Solid state lasers**

  Solid-state lasers are lasers based on solid-state gain media such as crystals or glasses doped with rare earth or transition metal ions. Solid-state lasers may generate output powers between a few milli watts and (in high-power versions) many kilowatts. The first solid-state laser – and in fact the first of all lasers – was a pulsed ruby laser, demonstrated by Maiman in 1960(Maiman, 1960).

- **Liquid lasers**

  A Liquid lasers use an organic dye in liquid form as their gain medium. They are also known as dye lasers and are used in laser medicine, spectroscopy, birthmark removal, and isotope separation. One of the advantages of dye lasers is that they can generate a much wider range of wavelengths, making them good candidates to be tunable lasers, meaning that the wavelength can be controlled while in operation(Fraser, 2022).

- **Gas lasers**

A gas laser is a laser in which an electric current is sent through a gas to generate light through a process known as population inversion. Examples of gas lasers include carbon dioxide (CO_2) lasers, helium–neon lasers, argon lasers, krypton lasers, and excimer lasers.

Gas lasers are used in a wide variety of applications, including holography, spectroscopy, barcode scanning, air pollution measurements, material processing, and laser surgery.CO2 lasers are probably the most widely known gas lasers and are mainly used for laser marking, laser cutting, and laser welding(Fraser, 2022).

B) Depending on wave length

Hard lasers- comes in infrared spectrum (> 700 nm)

E.g.: CO_2;Nd: YAG; Argon laser

Soft lasers – comes in UV (140-400nm) and visible light (400-700nm) spectrum

E.g.: HeNe, Diode laser

C) Based on safety procedure

- Class 1: safe under all conditions (fully enclosed system) – E.g.: Nd: YAG laser. Laser used in dental laboratory.
- Class 2: Output is 1 mm- visible low power laser- Visible red aiming beam of a surgical laser.
- Class 3A: Visible laser above 1 milli watt- No dental examples.
- Class 3B: Upper continuous power output limit is 0.5 w- Low power diode laser used for biostimulation. Direct viewing is hazardous to the eye.
- Class 4: Output excess of class 3B and are used for cutting and drilling- All lasers used for oral surgery, whitening and cavity preparation. Direct or indirect viewing is hazardous to the eyes (Ballal et al., 2013).

2.8 Laser Interaction with Biologic Tissue

The optical properties of tissue elements determine the type and extent of the tissue reaction through the process of absorption, transmission, reflection and scattering of the laser beam. The extent of the interaction of laser light as a form of radiant energy with tissue will generally be determined by two dependent variables, i.e. specific wave length of the laser beam and optical characteristics of the target tissue(Dederich, 1993).

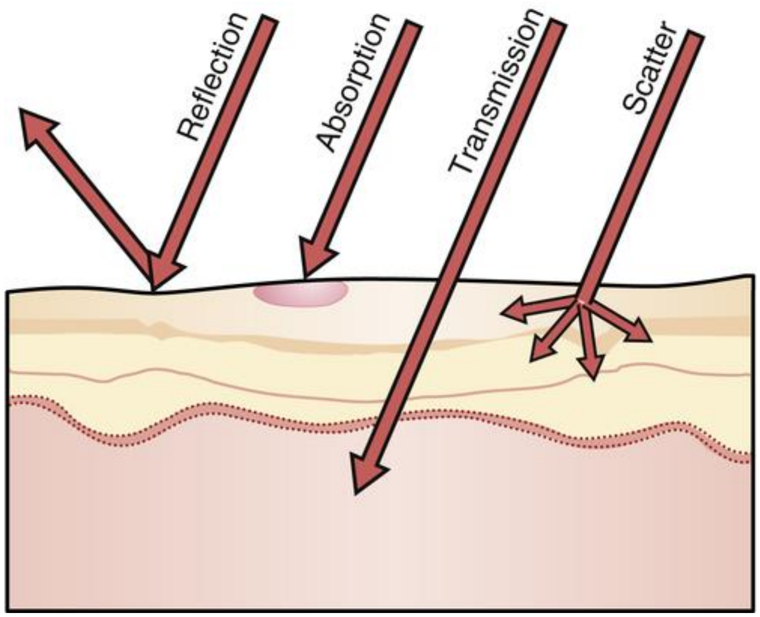

Figure 2.2 Four potential laser-tissue interactions (dentistry, 2015).

2.9 Optical properties of tissues

The laser energy interacts with the tissue in four ways: First, some of the incident beam can be reflected off the surface without the light energy entering or interacting with the tissue. Second, some of the light can be transmitted through the tissue. Third, some of the light can be absorbed by a component of the tissue. Fourth, the remaining light can penetrate and scatter the tissue without producing a perceptible effect on the tissue. In most cases, the degree of interaction is proportional to the degree of absorption of the particular wavelength by the tissue. Spectrographic analysis of oral hard tissues such as enamel, dentin, bone and synthetic hydroxyapatite show that the absorption, transmission and dispersion spectrographs are different for these four materials (Ballal et al., 2013).

2.10 Tissue Effects of Laser Irradiation

When radiant energy is absorbed by tissue, four basic types of interactions takes place:

A) Photo chemical interactions

B) Photo mechanical interactions

C) Photo thermal interactions

D) Photo electrical interactions

Photo chemical interactions include biostimulation which describes the stimulatory effects of laser light on biochemical and molecular processes that normally occur in tissues. Photo mechanical interactions include photo disruption or photo disassociation which is the breaking apart of structures by laser light and photo acoustic interactions which involve the removal of tissue with shock wave generation. Photo thermal interactions manifest clinically as photo ablation or removal of tissue by vaporization and super heating of tissue fluids, coagulation and homeostasis and photo pyrolysis or the burning away of the tissue.

Photo electrical interactions include photo plasmolysis in which tissues are removed through the formation of electrically charged ions. Generally, laser light of relatively low intensity and long duration are less destructive and include their effects at the cellular or molecular level, producing photo chemical effects (Ballal et al., 2013).

2.10 Tissue Thermal Properties

The medical laser applications are defined by the type of interaction between laser light and tissues. Knowledge of laser-tissue interaction can help clinicians and surgeons to select the optimal laser systems and to modify the type of their therapy. There for the knowledge about the laser-tissue interaction is the base to understand the interplay of laser light and its effect on tissues(Ansari et al., 2013), this can be reviewed in terms of :

2.10.1 Heat effects

The increase in body temperature leads to various effects such as hyperthermia, coagulation and other irreversible effects on tissues. When the temperature rises, the initial effect is hyperthermia. The typical range of 40-50 degrees Celsius is called the hyperthermia domain, where some molecular bonds are destroyed and the membrane is altered. The reduction in enzymatic activity is also observed. However, in this temperature range, the effects are reversible. At temperatures around 60oC, denaturation of proteins and collagen occurs, which leads to coagulation of tissue and can necrosis cells. Various optical treatments such as LITT and hair removal target temperatures above 60°C. At higher temperatures, the chemical concentration equilibrium is destroyed as the permeability of the cell membrane increases. The evaporation of water takes place at 100 °C.

Vaporization is sometimes referred to as a thermo-mechanical process because during the vaporization phase the temperature of the tissue is not changed and gas bubbles form. The propagation of these bubbles, accompanied by the change in their volume, causes the thermal decomposition of the tissue fragments . When all the water molecules are vaporized, carbon atoms are released and the adjacent tissue turns black and smoke comes out of the skin. this stage called carbonization. Finally beyond 300°C melting might occur (Ansari et al., 2013).

2.10.2 Heat transport

The laser energy can be absorbed by targets such as water, melanin and blood. This absorbed energy leads to an increase in tissue temperature. This energy can be taken as a source of thermal energy. The heat source S(r,z,t) within the exposed tissue is a function of the absorption coefficient a and the laser intensity I(r,z,t). In this regard, thermal conduction and thermal convection are important because they transfer thermal energy within the tissue. The physics of heat transfer is complicated; therefore we only explain some important results. Before defining the relaxation time, the of thermal penetration depth, which is another critical parameter to consider, is defined as :

Z therm (t)=$\sqrt{4kt}$ (1)

Z therm (t) : is the distance at which the temperature has decreased to 1/e of its peak value. In this equation κ is called temperature conductivity and its value is approximately the same for water1.4×10−7 m^2/s (Ansari et al., 2013). Thermal penetration depth can be defined as a distance in which the temperature decreases to 63% of its maximum value. This table expresses the thermal-temporal response of water; one shall keep in mind that heat diffuses in water up to approximately 0.7 micron within 1.0 microsecond. As it was expressed in reference number 1, the penetration depth, defined as L =1 a , is a distance in which the intensity of laser has decreased to 63% of its peak value(Ansari and Mohajerani, 1970). Experiments show that the relaxation time (relax τ) of water at the absorption peak which is for a wavelength near 3 microns is 1.0 microsecond. If the laser pulse duration τ is smaller than the relaxation time(relax τ <τ), the thermal energy cannot diffuse to the penetration depth; therefore thermal effects can be negligible. Heat can be diffused up to the optical penetration depth when relax τ >τ , hence thermal effects or damages are possible. The criterion s relax τ =1 μ is useful for wavelength of 3.0 micron, however for visible laser light relax τ is larger than 270 hours, This is not extraordinary, because water is transparent for visible laser light. Relaxation time for near infra-red (NIR) laser is smaller than 1.0 millisecond (Ansari et al., 2013).

heat conduction and heat convection are important means of heat transfer, this represented in the tissues by blood perfusion, It is worth stating that this perfusion rate is negligible in the first approximation, but for long exposure or LITT it has a significant role. Heat conduction can be stated as following:

$$J = -k\nabla T \qquad (2)$$

In this equation k is called heat conductivity and is expressed in units of W/mK . J is called heat flow. The value of heat conductivity at 37oC is 0.63 W/mK(Ansari et al., 2013).

2.11 Laser Delivery Systems

The beam of laser light should be able to be delivered to the target tissue in a manner that is ergonomic & precise.

Two delivery systems are used in dental lasers:-

One is flexible hollow tube that has interior mirror finish:

The laser energy is reflected along this tube & exists through a hand piece at the surgical end with the beam striking the tissue in a non-contact fashion. An accessory tip of sapphire or hollow metal can be connected to the end of the wave guide for contact with the surgical site.

Second delivery system is a glass fiber optic cable:

This cable comes in different diameters ranging from 200-1000 μm. The fiber fits snugly into a hand piece with the bare end protruding or with an attached sapphire or glass like tip. This system can be used in contact or non-contact fashion. Most of the time it is used in contact fashion.

Clinically a laser used in contact fashion can provide easy access. In non-contact fashion, the beam is aimed at the target at same distance away from it. But the disadvantage of this is lack of tactile sensation. In either mode the beam is focused by lenses within the laser itself. The laser device can emit the light energy in one of three basic modes.

Continuous wave: Beam is emitted continuously as long as the device is activated by pressing the foot switch.

Gated pulse mode: There are periodic alterations of the laser energy being on and off. (duration being millisecond)

Free running pulsed mode: Here large peaks of laser energy are emitted for short time (micro seconds) followed by a relatively long time in which laser is off. The important principle of any laser emission mode is that, the laser energy strikes the tissues for a certain period of time producing a thermal interaction. Hence we should see that, while using lasers, there shouldn't be any irreversible thermal damage to the target tissue as well as the surrounding tissue. If more heat is produced, it leads to delayed healing and postoperative discomfort. Hence a gentle air stream or an air current can be used to keep the area cool (Ballal et al., 2013).

2.12 Laser effects on dental hard tissues

Many effects of laser on hard dental tissues has been observed micro and macroscopically (Stubinger et al., 2008), these effects include :

2.12.1 Thermal effect

The thermal effect is the thermal vaporization of tissue by absorbing infrared laser light. The laser energy is converted into thermal energy or heat which destroys the tissue structures. The laser beam couples to the tissue surface, and this absorption leads to a heating with denaturation at about 45°C to 60°C. Above 60°C coagulation and necrosis can be observed accompanied by a desiccation of the tissue. At 100°C the water inside the tissue vaporizes. Carbonization and later pyrolysis (73wc) with vaporization of the bulky tissue terminate the thermal laser tissue interaction. The laser light will be absorbed and converted to thermal energy by stimulating the lattice vibrations of the tissue molecules. This leads to a heating of the surrounding tissues to a boiling of water followed by carbonization and tissue removal. Damage to the adjacent tissue is manifested by massive zones of carbonization, necrosis and thermally induced cracks.

2.12.2 Mechanical effect

High energetic and short pulsed laser light can lead to a fast heating of the dental tissues in a very small area. The energy dissipates explosively in a volume expansion that may be accompanied by fast shock waves. These waves can lead to very high pressures so that adjacent tissue will be destroyed or damaged. To avoid micro cracks in dental tissues, the maximum laser energy density of all laser systems must be kept below a certain threshold.

2.12.3 Chemical effect

The basis of the photochemical effect is the absorption of laser light without any thermal effect which leads to an alteration in the chemical and physical properties of the irradiated tissues.

2.12.4 Thermomechanical effect

Due to the good absorption of laser in water as well as in hydroxyapatite, the laser radiation leads to fast heating of water inside. In the mineralized matrix there is an explosive volume expansion. In dentin, no cracks are seen, but more thermal damage like carbonization and necrosis are found. In enamel, cracks are always found.

2.13Low-level laser therapy (LLLT)

Low-level laser therapy (LLLT) is a new branch of medicine that uses low-power lasers on living tissues to stimulate and improve cell function or relieve pain. Several studies have confirmed the reduction of pain and swelling and improvement of revascularization and wound healing(Eshghpour et al., 2017, Farivar et al., 2014, Holanda et al., 2017, Moosavi et al., 2016, Sardari and Ahrari, 2016).It is suggested that low-level laser therapy (LLLT) aids in the reduction of pain and the inflammatory process and enhances wound healing without any indication of side effects(Elson and Foran, 2015, Obradovic et al., 2009, Qadri et al., 2005, Sun and Tuner, 2004).

Low-level laser therapy, or laser photobiostimulation, is a form of phototherapy that uses coherent, monochromatic light on injuries and lesions to promote healing (Carroll et al., 2014). It is a non-thermal laser and operates in a wavelength range of 630-980 nm with typical irradiance of 5 mW/cm^2 to 5 W/cm^2 and generated by devices with as little power as 1 mW, and up to 10 W with either pulsed or continuous waves. The main absorber for these wavelengths are hemoglobin and melanin(Smith, 2007).

2.13.1 History and development

The roots of light therapy can be traced back to when our ancestors practiced heliotherapy, which gradually developed into actinotherapy and photomedicine (i.e., the use of UV radiation for sterilization). In 1903, Finsen was awarded the Nobel Prize for developing the carbon arc lamp incorporating lenses and filters for the treatment of lupus vulgaris (Sun and Tuner, 2004).

Subsequently, actinotherapy was used for treating TB and open wounds. In 1919, x-ray evidence led to the employment of ultraviolet radiation in the treatment of rickets. actinotherapy additionally stimulates the wound healing of ulcers, boils, and carbuncles and is employed in the treatment of acne vulgaris and neonatal jaundice and for pain relief. Nevertheless, its potential carcinogenic side effects limit its usage(Sun and Tuner, 2004).

After the first laser was developed in 1960 by Theodore Maiman in Los Angeles, there was a rapid development and interest in laser research. During the late 1960s in Budapest, Mester initiated studies on the possible carcinogenic effects of the low-power ruby and HeNe lasers. The mice he used in the study regrew hair faster than the controls, and the laser had no carcinogenic effect on the experimental group(Mester et al., 1971).Mester used this therapy for 875 patients with open wounds where conventional therapies had failed and had a success rate of 85%. Early experiments confirmed that the dosage follows the so-called Arndt-Schultz law: Too small a dose gives no effect, there is a therapeutic window within a certain dose range, and doses over that range are inhibitory (Mester et al., 1985).

In the 1980s, clinical applications of the LLLT started to appear (Sun and Tuner, 2004). Low-level lasers started gaining popularity in Europe, Asia, South America, and Australia, where the less-expensive and higher-powered output (30 mW or less) semiconductor (GaAlAs or GaAs) devices were used. In the 1990s, increasingly powerful lasers at reasonable prices delivering higher doses proved to be more effective. The most recently FDA-approved treatment of carpal tunnel syndrome and ‘‘minor chronic neck and shoulder pain of musculoskeletal origin’’ has also proved to have a positive effect on the awareness and development of the therapeutic laser therapy (Sun and Tuner, 2004).

2.13.2 Terms and definitions

The names to identify and differentiate therapeutic lasers from surgical lasers include soft, cold, low-intensity laser therapy, and LLLT. Therapeutic lasers are classified as class III medical devices, and surgical lasers are classified as class IV. Some phrases and phenomena describing the biologic effects of the therapeutic lasers are laser photobiostimulation, or biostimulation. In addition to the stimulating effect, the cellular effects also include bio inhibition, which can increase and decrease the physiologic functions to reach normalization. A more appropriate designation of the phenomenon might be laser photobiomodulation or laser bio activation. The phrase ‘‘therapeutic laser’’ has also been used to suggest the purpose and intent of the treatment (Basford et al., 1999, Zeredo et al., 2003).

2.12.3 Equipments

LLLT benefits can be performed with various wavelengths and units with different outputs. Usually the therapeutic window for sub thermal tissue interaction is 1 to 500 mW, but surgical lasers can be defocused and used as a ‘‘low level’’ laser (Basford et al., 1999, Zeredo et al., 2003). The first working laser, presented at a press conference arranged at the Hughes Aircraft Laboratory in Los Angeles on 7 July 1960, was a ruby laser (a solid-state laser using a single, rod-shaped ruby crystal). It emitted pulsed light at a wavelength of 694 nm. The ruby laser was also the first to be used in biostimulatory research in the mid-1960s(Sun and Tuner, 2004).Among its successors was the HeNe (Helium-Neon) laser, a gas laser emitting at 632.8 nm with a power output of 1 to 5 mW. The HeNe laser was used predominantly in Eastern Europe and China in the mid to late 1970s (Sun and Tuner, 2004).

The most popular lasers are relatively inexpensive diode units that were developed in the 1980s. The GaAs (gallium-arsenide; 904 nm) diode laser was developed in the early 1980s and was typically 1 to 4 mW. Pulse-train modulated GaAs lasers entered the market in the late 1980s. The GaAlAs (gallium-aluminum-arsenide; 780–890 nm) was developed in the late 1980s. It originally was designed as a 10- to 30-mW unit but since in the late 1990s has been featured up to 500 mW. The InGaAlP (indium-gallium-aluminum-phosphide; 630–700 nm) diode lasers were developed in the mid-1990s. Typically 25 to 50 mW, they offer an alternative to the HeNe laser for surface wound healing. Combination probes of two laser wavelengths or one or more laser diodes with LEDs of various wavelengths may be made as ‘‘cluster probes.’’ The effect of merging an incoherent LED with the laser requires further research to determine its effectiveness (Sun and Tuner, 2004).

Dental therapeutic lasers are usually the size of an electric toothbrush and come with a attachable intraoral probe shaped like the wand used in composite curing light units . The power of the GaAlAs lasers should not be less than 100 mW to obtain the desired biologic effect in a reasonable time.(Sun and Tuner, 2004).

2.12.4 Dosage and calculations

Although there is a wide therapeutic window in laser therapeutic applications , Although laser therapy offers a wide therapeutic window, an appropriate dose is essential (Bjordal et al., 2001). To calculate the dose (energy density), the given energy is calculated as mW per seconds (e.g., 100 mW×10 seconds = 1000 mJ = 1 J). The dose is calculated by dividing the energy with the irradiated area. If this area is 1 cm^2, the calculation is 1/1 = 1 J/ cm^2. If the irradiated area is 0.25 cm^2, the calculation is 1/0.25 = 4 J/cm^2. A reasonable power density (mW per cm^2) is necessary to trigger biologic effects, so low output cannot be fully compensated by longer exposure, and the depth of the treatment target site must be considered. Dose recommendations in seconds or minutes can be made only if the characteristics of specific laser are known. The amount of tissue between the laser probe and the target tissue and the type of tissue must be considered. For example, laser energy is more easily transmitted through mucosa and fat than through muscle. Hemoglobin and other pigments are strong absorbers of laser light and therefore require increased dosage. Penetration can be improved by using pressure, moving the laser closer to the target, or inducing a partial ischemia in the area. The skin coloration must also be considered because melanin is a strong absorber of light(Sun and Tuner, 2004).

Contact mode is needed for all applications with one exception. Treating an open wound requires a 2- to 4-mm separation distance between the laser and the target tissue. When contacting dental structures, some fluid might be needed to ensure full contact between the probe and the surface to minimize loss of energy. The following are some suggested treatment dosages: 2 to 3 J/cm two or three times a week on gingival tissues, 4 to 6 J/cm^2 two or three times a week on muscles, 6 to 10 J/cm2 once or twice a week on a TM joint, and 2 to 4 J/cm^2 directly on the tooth or indirectly above the apex or osseous structure.

2.12.4 Mechanisms of low level laser therapy

The principle of using LLLT is to supply direct biostimulative light energy to the body's cells. Cellular photoreceptors (e.g., cytochromophores and antenna pigments) can absorb low-level laser light and pass it on to mitochondria, which promptly produce the cell's fuel, ATP(Zhang et al., 2003). The most popularly described treatment benefit of LLLT is wound healing. From the studies of Mester et al (Mester et al., 1971), the electron microscope examination showed evidence of accumulated collagen fibrils and electrondense vesicles intra-cytoplasmatically within the laser-stimulated fibroblasts as compared with untreated areas. Also, the measurement from the incorporation of 3H-thymidine showed accelerated cell reproduction and increased prostaglandin levels after irradiation. Increased microcirculation can be observed with the increased redness around the wound area; during the initial treatment stage, the patient can feel the transient pin-prickling sensation, which is thought to be evidence of the accelerated wound healing (Sun and Tuner, 2004).

The mechanisms of action underlying the analgesic effects remain unclear, despite the implicit treatment benefits. There is evidence suggesting that LLLT may have significant neuropharmacologic effects on the synthesis, release, and metabolism of a range of neurochemicals, including serotonin and acetylcholine at the central level and histamine and prostaglandin at the peripheral level (Sun and Tuner, 2004). The pain influence has also been explained by the LLLT effect on enhanced synthesis of endorphin, decreased c-fiber activity, bradykinin, and altered pain threshold(Honmura et al., 1992, Laakso et al., 1994).

Skepticism surrounds the analgesic effects due to conflicting results, obvious placebo potential, and dominating subjective findings. This is an important field for research and investigation (Sun and Tuner, 2004).

The most recognized theory to explain the effects and mechanisms of therapeutic lasers is the photochemical theory. According to this theory, the light is absorbed by certain molecules, followed by a cascade of biologic events (Sommer et al., 2020). Suggested photoreceptors are the endogenous porphyrins and molecules in the respiratory chain, such as cytochrome c-oxidase, leading to increased ATP production(Sun and Tuner, 2004).

2.12.5 Clinical applications of low-level laser therapy

By understanding the basic cellular effects of the lasers and the intended treatment goal of reducing inflammation, accelerating the healing process, and providing pain relief, the general principles of application for various clinical, conditions become clear. Beneficial treatment effects have been applied in dermatologic conditions such as wounds(Garavello-Freitas et al., 2003, Sun and Tuner, 2004)

2.12.5 Biological Effects of Low-Level Laser Therapy

Low Level Laser therapy (LLLT) is the application of light to a biologic system to promote tissue regeneration, reduce inflammation and relieve pain. Unlike other medical laser procedures, LLLT does not have an ablative or thermal mechanism, but rather a photochemical effect which means the light is absorbed and cause a chemical change(Huang et al., 2009). The reason why the technique is termed low level is that the optimum levels of energy density delivered are low and it is not comparable to other forms of laser therapy as practiced for ablation, cutting, and thermal tissue coagulation(Hamblin and Demidova, 2006). The first law of photobiology explains that for a low power visible light to have any effect on a living biological system, the photons must be absorbed by electronic absorption bands belonging to some molecular photo-acceptors, which are called chromophores (Sutherland, 2002). The effective tissue penetration of light at 650 nm to 1200 nm is maximized. The absorption and scattering of light in tissue are both much higher in the blue region of the spectrum than the red, because the main tissue chromophores (hemoglobin and melanin) have high absorption bands at shorter wavelengths and tissue scattering of light is higher at shorter wavelengths. Water strongly absorbs infrared light at wavelengths greater than 1100 nm. Therefore, the use of LLLT in animals and patients almost exclusively utilizes red and near-infrared light (600-1100 nm) (Karu and Afanas'eva, 1995).

2.12.5.1 Mitochondrial Respiration and ATP

The current research about the mechanism of LLLT involves mitochondria (Huang et al., 2009). Cytochrome c oxidase (Cox) is a multicomponent membrane protein that contains a binuclear copper center (CuA) along with a heme binuclear center (a3-CuB), both of which facilitate the transfer of electrons from water soluble cytochrome c oxidase to oxygen. It is a terminal enzyme of the electron transport chain and plays a vital role in the bioenergetics of a cell(Srinivasan and Avadhani, 2012). It was proposed that Cox is the primary photo acceptor for the red-NIR range in mammalian cells because absorption spectra obtained for Cox in different oxidation states was found to be very similar to the action spectra for biological responses to light(Karu and Kolyakov, 2005).

The absorption of photons by Cox leads to electronically excited states, and consequently can lead to quickening of electron transfer reactions(Yu et al., 1997). The light induced increase in ATP synthesis and increased proton gradient lead to an increasing activity of the Na^+/H^+ and Ca^{2+}/Na^+ antiporters, and of all the ATP driven carriers for ions, such as Na^+/K^+ ATPase and Ca^{2+} pumps. ATP is the substrate for adenylcyclase, and therefore the ATP level controls the level of cAMP. Both $Ca^{2+}$and cAMP are very important second messengers. Ca^{2+} regulates almost every process in the human body (muscle contraction, blood coagulation, signal transfer in nerves, gene expression)(Hamblin and Demidova, 2006). Therefore the photoactivation of terminal enzymes, like Cox, plays an essential function in the activation of the numerous biological cascade observed subsequently to laser irradiation(Farivar et al., 2014).

2.12.6 Nitric Oxide and low-level laser therapy

The activity of cytochrome c oxidase is inhibited by nitric oxide (NO) (Beltran et al., 2000, Brown, 2001). This inhibition can be explained by a direct competition between NO and O2 for the reduced binuclear center CuB/a3 of cytochrome c oxidase, and is reversible(Antunes et al., 2004). It was proposed that laser irradiation could reverse this inhibition by photo dissociating NO from its binding sites(Karu et al., 2005, Lane, 2006). Because this coordinate binding is much weaker than a covalent bond, this dissociation is possible by LLL. The dissociation of NO from Cox increases the respiration rate (Karu et al., 2005). Light can indeed reverse the inhibition caused by NO binding to cytochrome oxidase, both in isolated mitochondria and in whole cells(Borutaite et al., 2000). LLL can also protect cells against NO-induced cell death(Hamblin and Demidova, 2006).

2.12.7 Reactive Oxygen Species (ROS) and Gene Transcription

LLLT was reported to produce a shift in overall cell redox potential in the direction of greater oxidation (Karu, 1999) and increased ROS generation and cell redox activity have been reported (Alexandratou et al., 2002, Chen et al., 2011, Grossman et al., 1998, Lavi et al., 2003, Zhang et al., 2008). It has been proposed that the redox state of a cell regulates cellular signaling pathways that control gene expression. Modulation of the cellular redox state can activate or inhibit signaling pathways(Srinivasan and Avadhani, 2012). Several regulation pathways are mediated through the cellular redox state. Changes in redox state induce the activation of numerous intracellular signaling pathways, such as nucleic acid synthesis, protein synthesis, enzyme activation and cell cycle progression(Liu et al., 2005). These cytosolic responses may induce transcriptional changes. Several transcription factors have been recognized to regulate by changes in cellular redox state. Among them redox factor-1 (Ref-1)-dependent activator protein-1 (AP-1) (Fos and Jun), nuclear factor B (NFB), p53, activating transcription factor/cAMP-response element-binding protein (ATF/ CREB), hypoxia-inducible factor (HIF)-1 and HIF-like factor are the most important factors(Hamblin and Demidova, 2006). Based on the ability of LLLT to modulate cellular metabolism and alter the transcription factors responsible for gene expression, it has been found to alter gene expression (Byrnes et al., 2005).

2.12.8 Low-level laser and Gene Expression

The gene expression profiles of human fibroblasts irradiated by low-intensity red light show that the irradiation can affect the expression of many genes that belong to different function categories. Irradiation of LLL stimulates cell growth directly through regulation of the expression of genes related to cell proliferation and indirectly through regulation of the expression of genes related to cell migration and remodeling, DNA synthesis and repair, ion channel and membrane potential, and cell metabolism. Irradiation by red light also enhances cell proliferation by suppression of cell apoptosis(Zhang et al., 2003).

Chapter Three
Materials and methods

3.1 Study Design

Randomized double-blind clinical trial

3.2 Study Area

Khartoum state.

3.3 Study Population

Patients attending a private dental clinic in Khartoum

3.4 Study duration

From January 2022 to March 2022.

3.5 Inclusion Criteria

- The age range of the participants between 18 and 50 years.
- No history of underlying systemic disorders.
- No active treatment with antibiotics, steroidal and non-steroidal anti-inflammatory drugs within the past month.
- No sign of periodontal problems in the target teeth.

3.6 Exclusion Criteria

- The occurrence of a dry socket (alveolar osteitis) at follow-up appointments.
- Occurrence of trauma during the extraction process e.g. root fracture, bone removal or soft tissue damage.
- The presence of smoking habit, pregnancy or breastfeeding in females.
- Patients who had more than one tooth extraction at the same time.
- Any drugs or habits that may interfere with or delay the socket healing process.

3.7 Criteria for treatment delivery

After the completion of the dental extraction, the participants were divided into 2 groups, in which groupone will receive LLLT with a wavelength of 980 nm, and group two will receive LLLT with a wavelength of 650 nm. A peak. The laser emitted at an approximately 10 mm distance to the target area. The target areas were the lingual, buccal and occlusal surfaces of the extraction socket. The irradiation was performed for 30 seconds to each target area. Laser exposure was performed after 30 to 60 minutes of extraction (day 1) and 2 days later (day 3).

A visual analogue scale (VAS) was used to assess the degree of pain perceived by the patients. This scale ranged from 0 to 10 with 0 (the left side) representing no pain and 10 (the right side) indicating the most terrible pain. The patients were asked to mark the degree of pain perceived on the VAS at bedtime for 7 days following tooth extraction. The patients were recommended to take ibuprofen 400 mg in painful conditions and record the number of consumption.

3.8 Low-level laser therapy equipment

- PIOON medical laser S1 Triple wavelength laser (Wuhan PioonTech.Co.,Ltd)
  - The first group : treated with 980 nm infrared laser for 90 seconds with a peak power of 1 watt and total energy of 180 J .
  - The second group : treated with 650 nm visible red laser for 90 seconds with a peak power of 200 mW and total energy of 144 J .

3.8.1 Measurement of post-operative pain

To establish measuring post-operative pain, the study Assigned “The British Pain Society” questionnaire on a rating scale from 1 to 10 of which 1 as having no pain and 10 is the worst pain(Society, 2022)

3.8.2 Measurements of post-operative satisfaction

The patients are requested to document their overall satisfaction on sensation of discomfort on a visual-analogue-scale with 0% being totally unsatisfied and 100% being completely satisfied (Doan et al., 2017).

3.9 Sample Size

This is an experimental study in which 30 patients were enrolled.

3.10 Sampling technique

Systematic random sampling.

3.11 Ethical considerations

- Approval letter from the Ethical Committee of the University of Sudan was obtained prior to the condition of the study.
- The aim and the methods of the study were explained verbally to the participants.
- Participant approval and written consent was obtained.

Chapter Four
Results and Discussion

4.1 Results and Discussion

A total of 30 participants divided into two groups (15 participants in each group) as shown in **figure 4.1**. The first group (treated with 980 nm diode laser) contained 11 males (73.3 %), and 4 females 26.7%. In the second group (treated with 650 nm diode laser) 7 males (46.7%) and 6 females (53.3) as shown in **table 4.1**.

The mean and the standard deviation of Age for the first group (980 nm) was found to be (40.1± 12.8), and for the second group (39.9±14.1) as shown in **table 4.2**.

One participant experienced bleeding in the first group (treated with diode laser 980 nm) with a percentage of (6.7%), and one participants in the second group (treated with diode laser 650 nm) with a percentage of (6.7%) as shown in **table 4.3** .

Independent Sample's test was done to show the mean and standard deviation of pain level on day 1 for the first group(treated with diode laser 980 nm) , and was found to be (1.2 ± 1.0) with a p value of (0.001) which is statistically significant, and for the second group(treated with diode laser 650 nm), the mean and standard deviation was (3.8 ± 2.0) as shown in **table 4.3**. Independent sample's test also used to show the mean and standard deviation for the first and second group on day 3 and found to be (1.0 ± 0.7) and (2.3 ± 0.8) respectively as shown in **table 4.4** .

Chi square test was not statistically significant for bleeding in both groups. The overall satisfaction for the two groups in which chi square was used to show the percentage of the participants' satisfaction as shown in **table4.5**.Also independent sample's test performed and showed that there is statistically significant difference in the overall satisfaction of the first group compared to the second group as shown in table**4.10** and **4.11**.

Table 4.1 : The percentage of males and females participated in the study.

	Male	**Female**	**Total**
Group 980	11	4	**15**
	73.3%	26.7%	**100%**
Group 650	7	8	**15**
	46.7%	53.3%	**100%**
Total	**18**	**12**	**30**
	60%	**40%**	**100%**

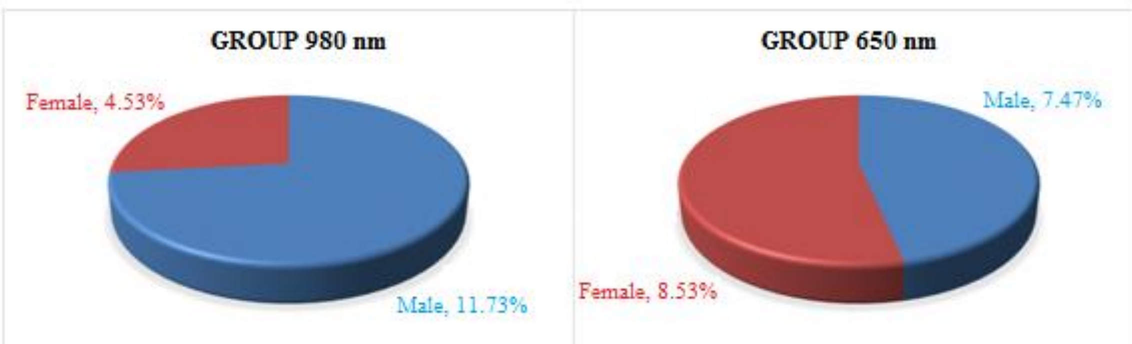

Figure 4.1: The distribution of the participants, *N*=30.

Table 4.2: The mean and standard deviation of age.

	N	Mean	SD
Group 980	15	40.1	12.8
Group 650	15	39.6	14.1

Table 4.3 : The mean and standard deviation of pain level at day 1.

	N	Mean	SD	P value
Group 980	15	1.2	1.0	0.001**
Group 650	15	3.8	2.0	

Table 4.4 : The mean and standard deviation of pain level at day 3.

	N	Mean	SD	P value
Group 980	15	1.0	0.7	0.001**
Group 650	15	2.3	0.8	

Table 4.5: Overall satisfaction of the first and second group in percentage.

	Not satisfied at all	Not satisfied	neutral	Satisfied	Very Satisfied	Total
Group 980	0	0	0	3	12	**15**
	0%	0%	0%	20%	80%	**100%**
Group 650	1	1	4	7	2	**15**
	6.7%	6.7%	26.7%	46.7%	13.3%	**100%**
Total	**1**	**1**	**4**	**10**	**14**	**30**
	3.3%	**3.3%**	**13.3%**	**33.3%**	**46.7%**	**100%**

Table 4.6: The mean and standard deviation for overall satisfaction

	N	Mean	SD	P value
Group 980	15	4.8	0.4	0.001**
Group 650	15	3.5	1.1	

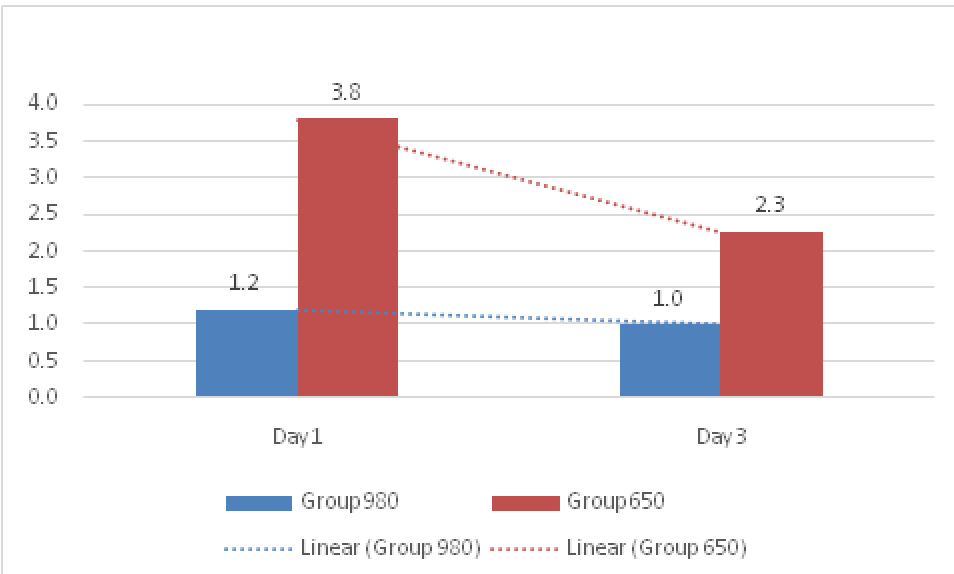

Figure 4.2: The linear relation of the first and the second group for pain level.

Table 4.7: Statistics of pain in day 1:

	Mean	Minimum	Maximum
Group 980	1.2	0	3
Group 650	3.8	1	7

Table 4.8: Statistics of pain in day 3:

	Mean	Minimum	Maximum
Group 980	1	0	2
Group 650	2.3	1	4

Table4. 9: Comparison of change in pain between day 1 and day 3 measured in reduction of visual analogue scale units.

	Group 980		Group 650	
	N	**%**	**N**	**%**
0 VAS unit	12	80	5	33.3
1 VAS unit	2	13.3	3	20
2 VAS unit	1	6.7	2	13.3
3 VAS unit	-	-	4	26.7
4 VAS unit	-	-	1	6.7
Total	**15**	**100**	**15**	**100**

The above table conclude that 80% of the population in the first group(treated with diode laser 980 nm) had maintained similar pain level during the first 3 days after extraction, while the remaining of the 980 group (20%) had a slight reduction of 1 and 2 VAS units. in the other hand, only 33% of the second group (treated with diode laser 650 nm) group showed no change in the VAS level during the first 3 days of extraction and almost 33% of the 650 group had to wait 3 days to experience 3 and 4 VAS units of pain reduction.

Table 4.10: The relation between satisfaction and level of pain in day 1:
Independent sample's test performed, *P value is significant

	Group 980	Group 650	P value
Very Satisfied	0.83	1.5	0.247
Satisfied	2.67	4.71	0.043*
neutral	-	3	-
Not satisfied	-	5	-
Not satisfied at all	-	4	-

Table 4.11: The relation between satisfaction and level of pain in day 3:

Independent sample's test performed.

	Group 980	Group 650	P value
Very Satisfied	0.83	1.5	0.164
Satisfied	1.67	2.57	0.114
neutral	-	2.25	-
Not satisfied	-	2	-
Not satisfied at all	-	2	-

Table 4.12: Comparison of pain level between males and females in the same study group Independent sample's test performed.

		N	Mean	SD	P value
Day 1	Male	11	1.3	0.9	0.662
	Female	4	1.0	1.4	
	Male	11	1.1	0.5	0.392
	Female	4	0.8	1.0	
Day 3	Male	7	4.1	1.3	0.542
	Female	8	3.5	2.5	
	Male	7	2.3	0.5	0.935
	Female	8	2.3	1.0	

Hence there is population matching between the 2 study groups, some may consider (gender) as confiding factor add to that the percentage of females was higher in group 650. The above table is intended to proof that there was no significant difference between males and females level of pain in the same study group. Regarding age as confounding factor, there was age matching between the two study groups.

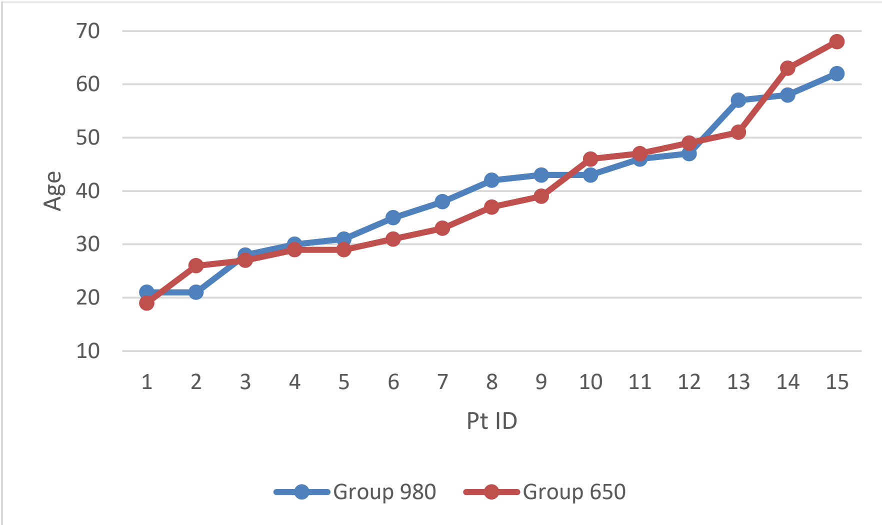

Figure 4.3: Age matching between the study groups.

The present study was performed to evaluate the effect of low-level laser therapy after tooth extraction as pain relief method. This randomized clinical trial carried on 30 participants which 15 patients received a 980 nm diode laser and the other group received a 650 nm.

The present study evaluated the pain level after tooth extraction and laser irradiation, and the overall satisfaction of pain relief after laser therapy by using the visual analogue scale (VAS).

A higher ratio of males to females in the present study (60% males) and (40% females), this in agreement with Farzaneh Ahrari et al (Ahrari et al., 2020) who reported significantly higher ratios of males to females.

Evaluation of pain for the study groups done using visual analogue scale (VAS), and it showed that both 980 and 650 nm wavelengths had an effect on pain reduction, with a statistically significant difference that 980 nm has a better effect on pain relief, on the other hand the mean and standard deviation of pain on day 1 and day 3 for the first group (group 980) was statistically significant, and this was in concordance with Farzaneh Ahrari et al (Ahrari et al., 2020).

The current study showed that the laser radiation with 980 nm wavelength has a better effect in relieving pain and this attributed the fact that the infrared radiation penetrate the tissues to about 2 to 3 cm, while the red laser of 650 nm has a low penetration depth of less than 10 mm according to Azam S Madani et al (Madani et al., 2014). On the other hand, the current study showed that 80% of the population in the first group (treated with diode laser 980 nm) had maintained similar pain level during the first 3 days after extraction, while the remaining of the 980 group (20%) had a slight reduction of 1 and 2 VAS units, compared to only 33% of the second group (treated with diode laser 650 nm) group showed no change in the VAS level during the first 3 days of extraction and almost 33% of the 650 group had to wait 3 days to experience 3 and 4 VAS units of pain reduction.

The present study showed that there is a significant difference between the satisfied populations of the 2 study groups, even though some of the group 650 showed to be satisfied about the procedure after the first day of extraction, their level of pain was significantly higher than their opponents in group 980. However, this only showed in the first day after extraction and there was no statistically significant difference on the third day after extraction, similar results found by Doan et al (Doan et al., 2017).

4.2 Conclusion

The present experimental study shows the importance of the Low-level laser therapy with infra-red light and visible light as a promising tool for pain management and healing acceleration after tooth extraction which in turn has a greater benefit for reduction of pain killers use which considered as a major cause for many systemic problems including renal failure (a major problem in the twenty first century). The results showed that there is statistically significant difference in pain reduction after extraction with the use of diode laser 980 nm, compared to diode laser 650 nm which showed a degree of pain reduction but no as much as the 980 nm laser.

4.3 Recommendations

In this study two diode laser (980 nm and 650 nm) were used to evaluate pain reduction, bleeding and post-operative satisfaction. The results showed significant benefits such in pain management and bleeding control However, further studies are needed to investigate other wavelengths specially in the visible spectrum, in which many reports showed a promising results.

Appendices

Appendix 1

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Appendix 2

PAIN RATING SCALE

(Arabic)

Title: .... Date:..

First Name:... Patient number:...

Surname:.. Clinic: ....

يرجى وضع علامة على المقياس أدناه لإظهار شدة ألمك.
صفر (٠) يعني لا يوجد ألم، و عشرة (١٠) تعني ألم شديد.

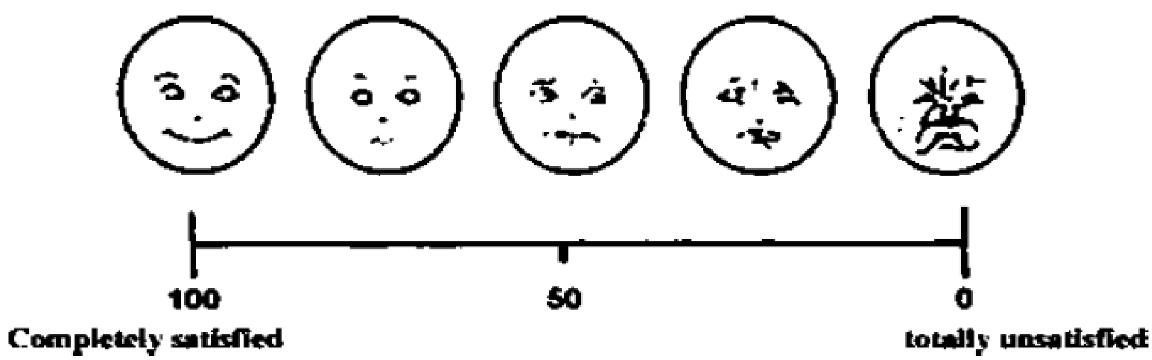
ما شدة ألمك الآن؟

٠ ١ ٢ ٣ ٤ ٥ ٦ ٧ ٨ ٩ ١٠

لا يوجد ألم ... ألم شديد

Appendix 3

A measure of overall satisfaction



Appendix 4

جامعة السودان للعلوم والتكنولوجيا

معهد الليزر

برنامج الدبلوم العالي لتطبيقات الليزر في طب الأسنان

استمارة موافقة مستنيرة للمشاركة في بحث بعنوان :

فعالية العلاج بالليزر منخفض المستوى في تقليل الألم وتسريع الشفاء بعد قلع الأسنان غير المضطرب

مقدمة:

ندعوك للمشاركة في هذه الدراسة البحثية حيث تشكل استمارة الموافقة هذه جزءاً من عملية قبولك و موافقتك على المشاركة والاستمارة مصممة بحيث تمنحك فكرة عامة عن هذه الدراسة وعن ما سيحدث لك اذا قررت المشاركة فيها. اذا رغبت في معرفة المزيد عن اي شيء ورد في هذه الاستمارة نرجو ان لا تتردد في الاتصال برئيس فريق البحث.

الغرض من البحث:

الغرض من هذه الدراسة هو تقييم مدى فعالية الليزر منخفض المستوى في تقليل الألم الناتج من خلع الاسنان.

الطريقة:

بعد قبولك للمشاركة في هذه الدراسة سيقيّم طبيبك حالتك لتحديد ما اذا كانت تطابق متطلبات الدراسة. وهذا يتطلب مراجعة تاريخك المرضي واخذ معلوماتك الشخصية وإجراء كشف طبي للأسنان و تعريض مكان الخلع لإشعاع ليزر منخفض المستوى.

الآثار الجانبية:

لا توجد اي آثار جانبية خطيرة تترتب على الدراسة سوى الشعور بدفء بسيط في مكان التعرض لضوء الليزر منخفض المستوى.

الفوائد الممكنة :

الفوائد المرجوة من هذه الدراسة هي تقييم مستوى خفض الألم المصاحب للخلع عن طريق الليزر والذي قد يكون بديلاً مستقبلياً لاستخدام المسكنات والتي لها اثار جانبية على المدى الطويل.

التكلفة/استعادة التكلفة:

لن تكلفك الدراسة اي رسوم نقدية.

انهاء المشاركة:

مشاركتك في هذه الدراسة عمل طوعي. ولن تؤثر في سير علاجك اذا قررت عدم المشاركة.

السرية:

لك كل الحق في التمتع بخصوصيتك ونؤكد لك في هذا السياق ان كل المعلومات التي تجمع في اطار هذه الدراسة ستظل سرية وللاستخدام العلمي فقط.

الاتصال بفريق الدراسة:

د/ محمد حسن الحاج العوض – اخصائي اول : امراض وجراحة اللثة وما حول السن

Senior specialist in Periodontics

رقم الهاتف: 0922161666

البريد الإلكتروني:

mohammed.hassan.elhaj@gmail.com

الموافقة:

اقر بالآتي:

1.قرأت المعلومات اعلاه وشرح لي كل ما بتعلق بهذه الدراسة البحثية.

2. لدي فرصة في طرح الاسئلة وقد تمت الاجابة على كل الاسئلة التي طرحتها.

3.مشاركتي في هذه الدراسة البحثية عمل طوعي.

4.يمكنني الانسحاب من هذه الدراسة في اي وقت دون ان يؤثر ذلك في امكانية حصولي على علاج بديل متوفر.

5.ادرك تماماً انه قد لا تكون لي فائدة طبية مباشرة من المشاركة في هذه الدارسة البحثية.

6. ستقدم نسخة موقعة من استمارة الموافقة هذه.

التوقيع:........................ التاريخ:.........................