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Diode Lasers (940 and 810) nm Cutting Efficiency of Oral Soft Tissue Assisted by External Chromophores: Histopathological Study

A Thesis Submitted to the Institute of Laser for Postgraduate Studies, University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master of Science in Laser / Dentistry

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

صدق اللَّهُ العظيم

(المجادلة 11)

Dedication

To my parents, wife and son ...
Thanks for love and support

Ali

Certification

I certify that this thesis was prepared under my supervision at the Institute of Laser for Post Graduate studies, University of Baghdad as a partial fulfillment of the requirements for the degree of Master of Science of Laser / Dentistry

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Abstract

Background: The diode laser is becoming widely used for surgical oral soft tissue procedures, due to approximate relation of infra- red wavelengths with absorption of oral soft tissue chromophores. Thermal damage is one of the undesirable effects that result from heating the tissue by laser.

Aim: In vitro histopathological evaluations of the effect of diode laser (940 or 810) nm on incision properties and thermal damage when incision assisted by external chromophores (erythrosine 3% or methylene blue 1%).

Materials and methods: 120 samples of dimensions (1.5 × 1 × 0.5) cm from six sheep tongues collected directly after animal sacrificing, divided into (A and B) groups. Group A, 60 samples incised using diode laser 940 nm at 2 W, C.W, initiated surgical tip. This group was subdivided into AI (20 samples) which incisions were made with normal saline, while AII (40 samples) the incisions were assisted with erythrosine stain 3%. Group B, 60 samples incised using diode laser 810 nm at (1.5, 2.5) W, C.W, initiated surgical tip. This group was subdivided into BI (10 samples, 1.5W) and BIII (10 samples, 2.5 W) which incisions were made with normal saline, BII (20 samples, 1.5 W) and BIV (20 samples, 2.5W) the incisions assisted with methylene blue 1%.

Results: The result showed highly significant difference in incision time with ($P<0.01$), high significant difference in depth and width of thermal damage ($P<0.01$) and high significant difference of incision margins regularity with ($P<0.01$) for all stained samples with (erythrosine 3% and methylene blue 1%) stains in both A and B groups.

Conclusion: Compared to laser cutting of unstained tissue, using the external chromophores can result in decreasing the cutting time, minimizing the thermal damage both vertically and horizontally and produce more regular incision margins.

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List of abbreviations

Abbreviations	Term
GIT	Gastro intestinal tract
UV	Ultraviolet
Nd:YAG	Neodymium doped Yttrium-Aluminum garnet
Er,Cr:YSGG	Erbium, chromium-doped yttrium scandium, gallium and garnet
C.W	Continuous wave
W	Watt (unit of power)
W/cm^2	Watt/square centimeter (unit of irradiance)
Sec	Second (unit of time)
PDT	Photodynamic therapy
ATP	Adenosine triphosphate
TNF	tumor necrosis factor
eV	Electron volt
OMF	Oral maxillofacial
GaAs	Gallium arsenide
W/W	Weight/weight
Red no.3	Erythrosine stain
TSH	Thyroid stimulating hormone
PH	Potential of hydrogen (scale of acidity and basicity of an aqueous solution
HIV	Human immunodeficiency viruses
SEM	Scanning electron microscope
OPMDs	Oral potentially malignant disorders
MB	Methylene blue
Ac	Alternating current

Hz	Hertz (unit of frequency)
H&E	Hematoxylin and Eosin
SD	standard deviation
SE	standard error
λ	Wavelength
υ	Frequency

Introduction and Basic concept

1.1. Introduction

Laser is being widely used in dental practice with subsequent improvements and innovations over time, as first successful application in dentistry in 1977 (Thomas and Isaacs, 2012).

The laser unique characteristics which are collimation, coherence and monochromatic radiation provides a special applications in medicine and surgical field, especially in oral and maxillofacial surgery (Souvik et al., 2014).

The diode laser has excellent incision performance on soft tissue with very good surgical and hemostatic action via sealing small lymphatic and blood vessels which result in minimize post-operative pain and edema due to absorption of laser energy by specific absorber that exist with the tissue (Mavrogiannis et al., 2004).

When the tissues or any surface are irradiated by laser light, the energy of laser beam can be reflected, scattered, transmitted or absorbed by the tissue and a specific interactions occurs. When laser transmitted through the tissue, there will be minimal or no absorption of laser energy and so minimal or no thermal effect can occur. The transmission depth inside the tissue based on laser parameter (wavelength, exposure time, delivery mode), and tissue properties which are optical, thermal and conductivity (Genovese and Olivi, 2010).

There is characteristic absorption for each tissue which is depends on the composition of tissue and its target chromophores content. For mammalian tissue the melanin, hemoglobin, water, and proteins are the basic chromophores exist. The hemoglobin and oxyhemoglobin are considered as the light receptors (chromophores) of the biological tissues present in the blood corpuscles which circulates in blood vessels, make them desired for diode laser absorption, which

result in very good coagulation and hemostasis effect. They are red in color and exist internally within the tissue presented as internal chromophores. When the laser beam absorbed by tissue, the laser energy is converted to heat and in order to obtain the desired thermal biological effect, the key is the selective absorption of specific laser wavelength by target chromophores (Douglas and Dederich, 1991).

1.2. Anatomy of oral cavity

The oral cavity is the first part of elementary canal which lies between the GIT mucosa and the skin, the anatomical boundaries of oral cavity are palate superiorly, muscular floor inferiorly, lips anteriorly, facial arches posteriorly, the cheeks, upper and lower teeth laterally which are shown in figure **(**1-1). (Alexander et al., 2019).

The mouth is composed of two parts, which are the oral cavity proper and vestibule. Normally the mouth is moist, lined with a mucus membrane and contains teeth. The lips marked the transition from mucous membrane to skin that cover most of body (Pocock and Gillian, 2006).

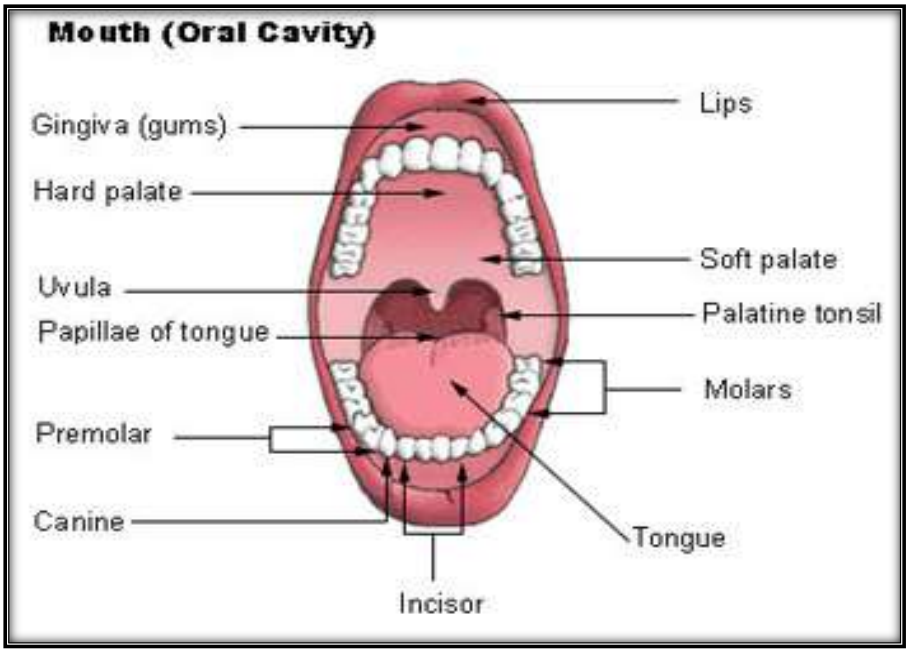

Figure (1-1) Anatomy of oral cavity (Pocock and Gillian, 2006).

1.2.1. Nerves supply

The innervations of the oral cavity are of two types:

1. Sensory innervation: The roof of the oral cavity is supplied by maxillary nerve branches (greater palatine and nasopalatine), and the floor is innervated by the mandibular nerve brunch (lingual nerve), the branch of the mandibular nerve (buccal nerve) is innervate the cheek (Dotiwala and Samra, 2020)

2. Motor innervation: The orbicularis oris muscle of lips and the buccinators muscle of cheek are innervated by branches of facial nerve (Saraswathi et al., 2006).

1.2.2. Blood supply

The pharynx and the oral cavity are supplied by branches of maxillary artery and external carotid artery (lingual artery, posterior auricular artery, ascending pharyngeal artery, facial artery, superficial temporal artery). The venous drainage is by many tributaries which are lingual, facial and pharyngeal veins (Mun et al., 2016).

1.2.3. Function of mouth (oral cavity)

The mouth has a significant role in drinking, speaking and eating. When there is a breathing obstruction through the nose which is the designed breathing way for human, the alternative way is through moth breathing as a temporary backup system. Infants are born with sucking reflex, they know instinctively sucking for feeding using their jaws and lips. The mouth also aids in food biting and chewing (Maton et al., 1993).

1.3. Oral mucosa

The oral mucosa represents the moist lining of oral cavity. It is formed by Papillary layer (stratified squamous epithelium) and Reticular layer (lamina propria), the two layers are separated by basement membrane and underneath is the submucosa (Squier and Brogden, 2011). Figure (1-2) shows the layers of oral mucosa.

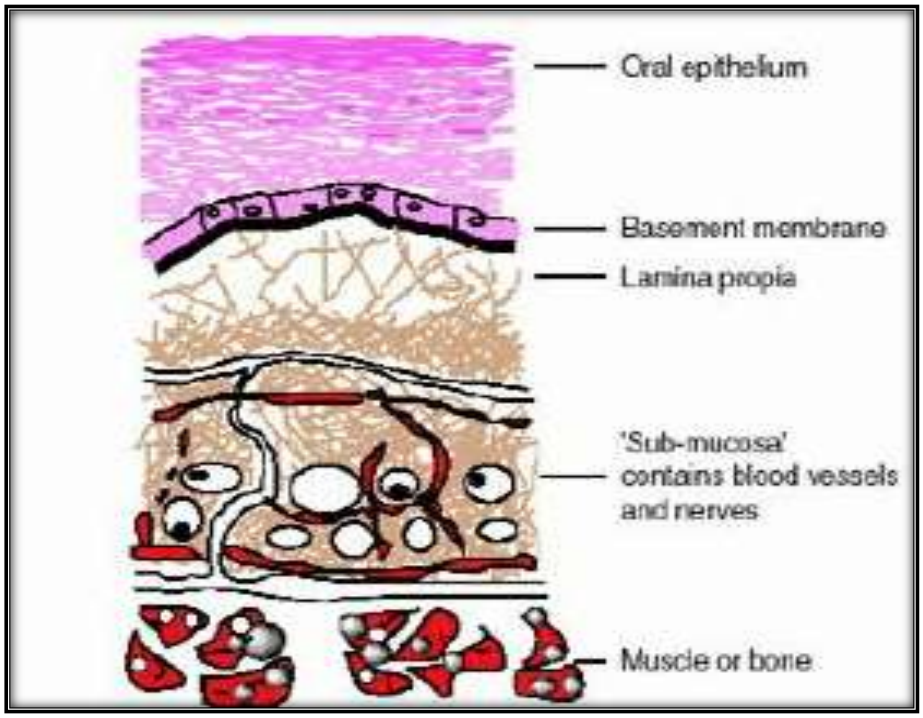

Figure (1-2). The layers of oral mucosa (Squier and Brogden, 2011).

1.3.1. Epithelium

The epithelium of oral mucosa is a soft, protective, continual, cell layer. The epithelium lines blood vessels and the organs outer surfaces throughout the body, also lines the inner surfaces of many internal organs cavities. The epithelium thickness is variables in various areas of oral cavity that shown in table (1-1), (Squier et al., 2001).

Table (1-1) Thickness of the epithelium of oral cavity (Squier et al., 2001).

Anatomic location	Thickness (μm)
Gingiva	± 329.8
Labial mucosa	± 364.4
Buccal mucosa	± 624.5
Ventral surface of tongue	± 373.0
Floor of mouth	± 135.3
Tongue dorsum	± 835.6

The oral mucosa composed of stratified squamous epithelium and it is of two types: (Pratt 2012)

1. Keratinized epithelium which present in gingiva, hard palate and dorsal surface of tongue, and consists of four layers:

A. Basal layer (Stratum basale)

B. Prickle layer (Stratum spinosum)

C. Granular layer (Stratum granulosum)

D. Keratinized layer (Stratum corneum)

2. Nonkeratinized epithelium which cover the internal part of ventral surface of tongue, cheeks, lips, soft palate, mouth floor. It consists of two deep layers and the outer layers named as intermediate and superficial layers.

1.3.1.1. Epithelial cells of oral mucosa

1. Keratinocytes

Are the first cell type found in the epidermis, the most outer layer of skin which formed 90% of epidermal skin cells in humans and present in some regions of keratinized epithelium of oral mucosa where keratinization occur, which is the discrimination of keratinocytes within the stratum granulosum into non vital squames to compose the stratum corneum (James et al., 2005).

2. NonKeratinocytes

I. Langerhans cells

It is composed of unique, racket-shaped organelles, a type II antigen processing cells, so they are function in the processing of antigenic material (Blauvelt et al., 1995).

II. Merkel cells

Merkel cells present in the basal layer of gingival epithelium and associated with the terminal axon. These cells along axon function like receptors for pressure and touch and are slowly adaptive (Boulais et al., 2007).

III. Melanocytes

These cells exist in the basal layer of gingival epithelium and are responsible for melanin pigments production. The nearer keratinocytes may get melanosomes that introduced by melanocytes (Feller et al., 2014).

IV. Lymphocytes, Leukocytes and mast cells

Lymphocytes, Leukocytes and mast cells are inflammatory cells. They could be found in the connective tissue and gingival epithelium during gingival inflammation. The gingival tissue during inflammation may contain mast cells bearing granule (Abe et al., 2000).

1.3.2. Lamina propria

It is a fibrous connective tissues layer, composed of a mesh of type I and III collagen and elastin fibers in some area. The fibroblasts are primary cells of the lamina propria and they responsible for the fibers production and extracellular matrix. The superficial layer of lamina propria consists of loose connective tissue along with blood vessels and nerve tissue. The lamina propria deeply consists of dense connective tissue layer with a high amount of fibers and between the lamina propria two layers there is the capillary plexus that supplies the nutrition for all mucosal layers and gives capillaries into the connective tissue papillae (Burkitt et al., 1993).

1.3.3. Basement membrane

It is a soft, flexible sheet-like extracellular matrix which acts as a stand for complex signaling and provides cell and tissue support. The basement membrane surrounded by epithelial tissues and the underlying connective tissue (Yurchenco P.D 2011).

1.3.4. Submucosa

It is a thin layer of irregular dense connective tissue that upholding the mucosa and link it to the muscular layer, according to the position in the oral cavity, if the submucosa present it may composed of loose connective tissue and

salivary glands or adipose tissue along with bone or muscle that overlying within the oral cavity (Kierszenbaum et al., 2012).

1.3.5. Types of oral mucosa

Depends on variable functions and structures, the oral mucosa is divided into three types:

1. Lining mucosa

The lining mucosa is a flexible and thin, covering the ventral side of tongue, cheeks and floor of oral cavity, the alveolar processes, the inner side of lips and the soft palate. Composed of thin non-keratinized epithelium in the cheeks, mouth floor, alveolar process and ventral side of the tongue (Field and Longman 2003).

I. Ventral Surface of the Tongue

It consists of mucosa, lamina propria and submucosa. The lining mucosa is composed of nonkeratinized epithelium, the submucosa contains muscle fibers (Susan 2008).

II. Cheeks

The cheeks are consists of mucosa, lamina properia, submucosa. The mucosa of cheeks composed of nonkeratinized stratified squamous epithelium, the submucosa composed of mixed glands (seromucous) and fat cells which are exist between muscle fibers. The mixed glands are considered the characteristic feature of the cheeks (Nanci, 2013).

III. Floor of mouth

It is a space with U-shape that aligned horizontally, located under the tongue. The floor of the mouth main structures are the geniohyoid muscles, the mylohyoid muscles, the sublingual glands, deep process of the submandibular glands along with the submandibular duct, the large veins, the sublingual and submental arteries, and their branches (La'porte et al., 2011).

IV. Alveolar process

It is the tooth socket lining, it is formed by compact bone with many holes where Volkmann canals transcend into the PDL from the alveolar bone and named as cribriform plate (Nanci, 2013).

V. Lips

The lips are lined by moist surface from inner side. It consists of nonkeratinized stratified squamous epithelium. The epithelium is linked to lamina propria via round seromucous glands. The vermilion border is a red border separate mucosal part from the skin of the lips and it is composed of a nonkeratinized epithelium that modified to keratinized epithelium (Chiego and Daniel, 2018).

VI. Soft palate

It is a mobile muscular flap that continues with the posterior part of the hard palate into the pharyngeal cavity. It is the separation area between the oropharynx and the nasopharynx. The epithelium of nasal part of soft palate is pseudostratified ciliated columnar, while the epithelium of oral part of soft palate is non-keratinized stratified squamous (Vishram, 2014).

2. Masticatory Mucosa

The masticatory mucosa lined the gingiva and hard palate which are exposed to the forces of mastication. It occupies 25 % of oral cavity, and it is composed of moderately thick epithelium and mostly orthokeratinized with area of parakeratinization may be seen (Susan, 2008).

I. Gingiva

The gingiva develops like a coalescence of the oral epithelium with reduced enamel organ of oral cavity. At time the tooth emerges into the gingiva, the cervicals of the teeth surrounds by oral epithelium and continue to the mucogingival junction. The gingiva attach to the tooth at the cervical area via

junctional epithelium. The floor of gingival sulcus is covered by the junctional epithelium (Lindhe et al., 2008).

II. Hard Palate

It forms the roof of oral cavity, composed of epithelium, lamina propria with no submucosa. The epithelium is keratinized stratified squamous (Nanci, 2005).

3. Specialized Mucosa

The specialized mucosa is the covering of 15% of the oral cavity including superior surface of tongue (dorsum). Although its function like masticatory mucosa but it is considered as specialized mucosa because of its high extensibility (Chiego and Daniel, 2018).

Tongue

The tongue is filling the oral cavity floor and is attached to the pharynx, styloid processes, hyoid bone and mandible by muscles. It is mainly formed by skeletal muscles and partly covered by mucus membrane. The tongue is consist of anterior two third (body) and the posterior one third (root) and they are separated by sulcus terminalis (Chiego and Daniel, 2018).

1.3.6. Functions of oral mucosa

1. Protection: A barrier against microbiological insult and mechanical trauma
2. Secretion: Secrete saliva which maintains the moisture of mouth.
3. Sensation: Its receptors that respond to pain, taste, touch, temperature and initiates reflexes like gagging, swallowing and salivation (Nanci, 2003).

1.4. Laser Basics

The laser word is an abbreviation for amplification of light by stimulation of emitted radiation. Based on theories of Einstein in 1916, according to which a photon can stimulate the emission of the similar photon. The laser was firstly invented in medicine by Maiman in 1960. The first use of lasers in dentistry was

made by Stern and Sognnaes (1964) and Goldman et al. (1965), describing the effect of a ruby laser on hard tissues in dentistry (Souvik et al., 2014).

1.5. Properties of laser light

1. Monochromaticity

It is the property of laser in which it produce a beam with single color according to the laser wavelength and where it lies in the spectrum (Mercer, 1996).

2. Coherence

The property that all laser waves are identical in the size and shape physically, which means that frequency and amplitude are identical for all the waves of photons that produce a special form of focused electromagnetic energy (Eichhorn 2014).

3. Collimation

This property means that all laser waves are parallel to each other which maintain the brightness of laser beam, but there will very little divergence over long distance (Myers, 1991).

1.6. Components of laser device

There are 3 basic components of each laser device, figure (1-3) shows the basic components of laser.

1. Active medium: it could atom, molecule or compound. The active medium could be gas like Co_2 laser, semiconductor like diode laser, solid crystal of yttrium aluminum garnet (YAG) like Er:YAG and Nd:YAG , or sometimes could be liquid (Taylor and Nick, 2000).

2. Pumping source : The pumping source could be either electric like electric circuit or optical source like flash lamp which is surrounding the active medium and is responsible for the excitation of atoms or molecules of active medium (Poehler and Walker, 2018).

3. Optical resonator: The resonator is either two reflective mirrors which are placed parallel to each other inside optical cavity at the same axis of active medium or could be two polished surfaces like in semiconductor laser. The action of optical resonator is by reflecting the waves forth and back inside laser cavity producing amplification (Orazio and David 2010).

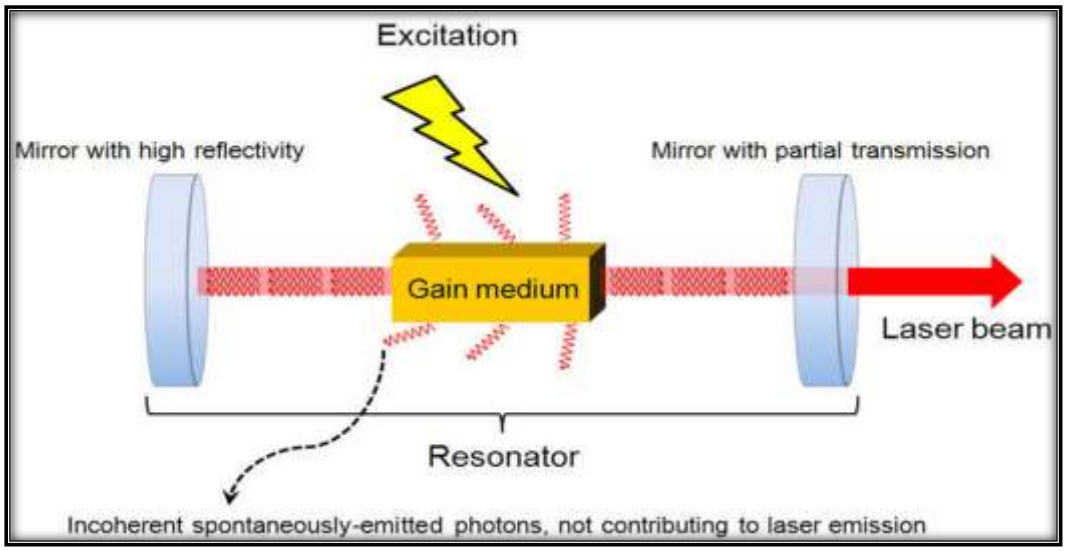

Figure 1-3 Basic components of a laser (Coluzzi, 2004).

1.7. Laser emission modes

1. **Continuous wave (CW)**:

The power of laser in this mode is stable without any fluctuation throughout the time (Reddy, 2017).

2. Pulsed mode which is divided into two types

A. Gated pulsed mode:

This mode is produced in laser device which operates with continuous mode via placing mechanical shutter interior to the of laser beam bathway which result in periodic alteration or fluctuation in laser energy as blinking of light, the pulse duration in this mode usually in microsecond (μsec), millisecond (msec) and etc (Coluzzi, 2004).

B. Free running mode:

It is also named as true pulsed, the true pulsed laser depend on pumping source action so the high peak energy of laser is delivered for short period then followed by long duration of laser off (Freitas and Simoes, 2015) .Figure (1-4) shows laser emission modes.

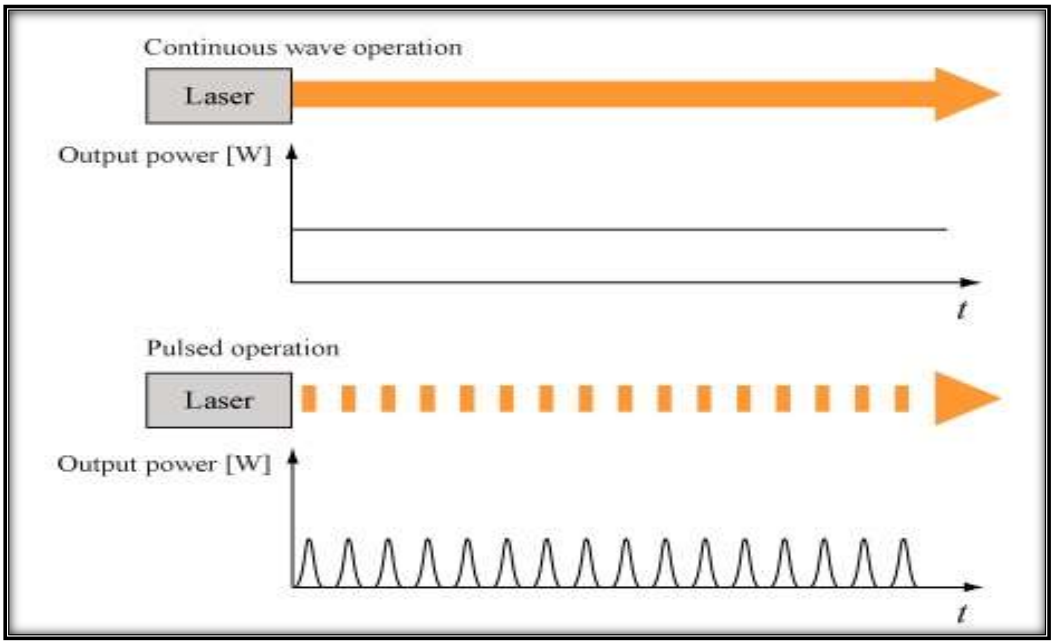

Figure (1-4) Laser emission modes (continuous and pulsed), (Malik and Chatra, 2011).

1.8. Laser Delivery Systems

For laser to be useful in clinical work, the energy of laser should be delivered efficiently to the target tissue.

The delivery systems includes**:** The hollow waveguides is a flexible tube contains reflecting internal surfaces, used with far and mid infrared laser. The articulated arms consist of mirrors at joints used with infrared, visible and UV lasers. The fiber optics used with visible and near infrared lasers. The fiber optic delivers laser energy to almost every site of mouth and to complicated root canal systems so it considers as the system of choice for most laser devices (George, 2009).

Dental laser can be used in focused or defocused modes, by using lenses the laser beam can be focused so can be used in incision and excision of tissues, while in defocused mode the laser energy is diverged over wider area and it is useful in coagulation and hemostasis. (Shimoda, 2013).

1.9. Laser parameters (Convissar, 2015).

It is very important to consider these parameters when using laser radiation which are:

I .Wavelength (λ): It is the distance at which the waveform repeats, usually measured in meter and is considered a very important characteristic of laser.

II. Frequency (υ): It is the number of wave oscillations per second and it is measure by hertz (Hz).

III. Pulse duration: it is the full width at half maximum of a single pulse of pulsed laser.

IV. Peak power: It is equal to energy in Joule/pulse duration

V. Average power: It is equal to energy in Joule*pulse repetition rate

VI. Pulse repetition rate: It is the how many number of pulses per second.

VII. Spot size: refers to the diameter of laser beam on the targeted tissue.

VIII. Power density (Irradiance): It is equal to power (w) per area of unit (W/cm^2)

IX. Energy density: It is equal to energy in joule per unit area (J/cm^2).

1.10. Laser tissue interaction

when laser beam hitting tissue there will be four behavior of tissue (absorption, reflection, transmission and scattering) which are depends on optical properties of tissues (coefficient of absorption, coefficient of scattering, thermal relaxation time, penetration depth) and laser parameters (wavelength, power, spot size, energy and etc.), (Dederich, 1993), figure (1-5) shows the different behaviors of tissue to laser radiation.

1. Absorption:

The laser energy absorption by tissue is depending on two factors which are tissue properties which is the presence of absorber or chromophore inside the tissue such as (water or pigments) and the wavelength that matching the tissue absorber (Parker S. and Convissar 2011).

2. Reflection

The reflection occurs when the laser beam is redirected away from target tissue surface without any influence on tissue. The redirected light may has a dangerous effect on unwanted site (Dederich, 1993).

3. Transmission

When laser energy passing through the tissue without any influence on tissue, that mean the tissue acting as transparent medium to the laser light like in Nd:YAG and diode laser when directing through the water (David et al 2015).

4. Scattering

It occurs when photon redirected inside the tissue, so lead to more absorption as a result of increase chance of interaction of chromophores with the wavelength. The undesirable damage caused by scattering is due to thermal transmission to neighboring tissue (Ansari and Mohajerani, 2011).

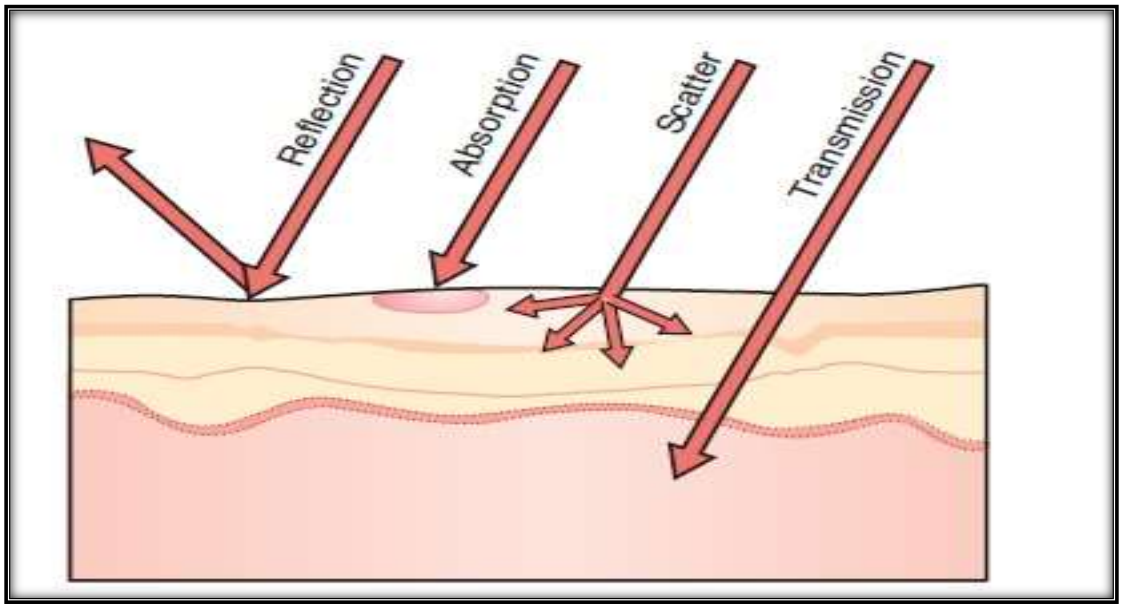

Figure (1-5) Different behavior of tissue to laser radiation (Convisar 2015).

1.11. Laser-tissue interactions mechanisms

The laser has different applications in medicine which are depends on how it is possible to induce etching or fragmentation of tissues, local necrosis and will determined based on tissue characteristics and laser parameters. There are two mechanism of interaction shown in figure (1-6). Different biological effects of laser on tissue according power density and exposure time are shown in figure (1-7), (Namour, 2011).

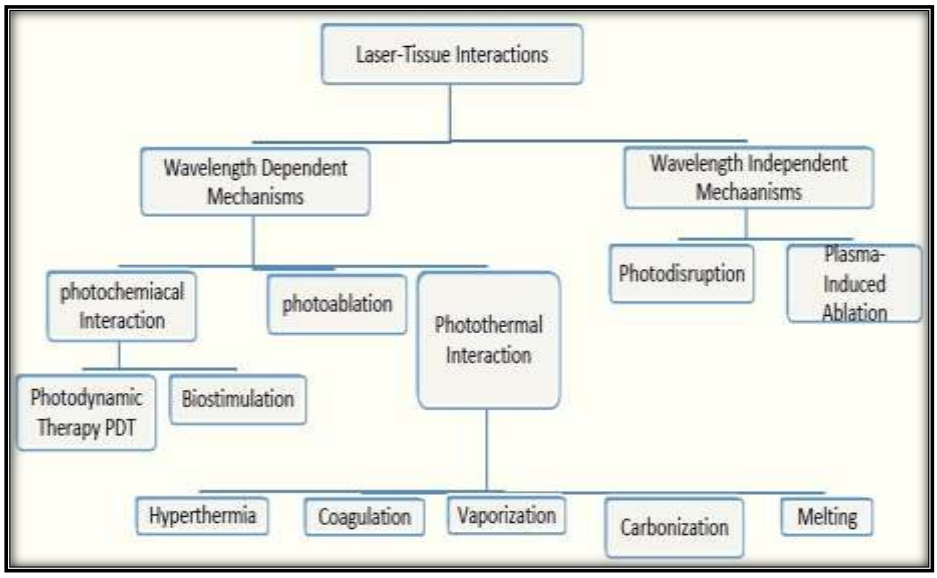

Figure (1-6) laser tissue interaction mechanism (Niemz, 2013).

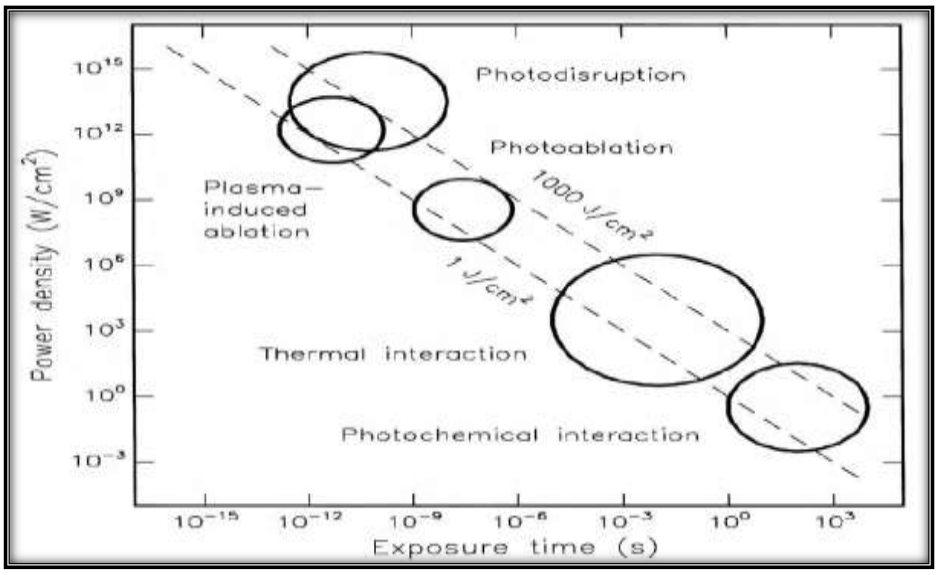

Figure 1-7 Map of laser–tissue interactions mechanisms. The relationship between power density and exposure time produces a various photobiological effects (Niemz, 2013).

1.11.1. Wavelength dependent mechanism

1. Photothermal interaction

At power density of $1–10^6$ W/cm^2 and exposure time of 1 msec–100 sec the photothermal effects appear. The local thermal elevation is the governing parameter of this interaction (Knappe et al., 2004). Table (1-2) shows the thermal effects of laser radiation on tissues.

The laser modes (C.W or pulsed) can induce photothermal effect in the tissue because the laser energy is converted to heat, no effect if the temperature elevation is within human body temperature or slightly above it, but irreversible cells damage will occur if the temperature elevation is above the crucial point in which the mechanism of cell repair by themselves is not work anymore, and the end result is the necrosis (Coluzzi, 2008). Figure (1-8) shows Relation between temperature duration and minimal critical temperature.

Table (1-2) Thermal effects of laser radiation on tissues (Alazzawi et al., 2011).

Temperature (°C)	Biological effect
37°C	Normal
45 °C	Hyperthermia which is the first thermal effect in which characterized by bond destruction and membrane alteration and its last for several minutes and at this time necrosis occur in significant percentage of the tissue cells.
50 °C	Reduction in enzyme activity, cell immobility
60°C	At this temperature level, proteins and collagen denaturation occur which lead to tissue coagulation and cells necrosis; it is obvious macroscopically as visible tissue paling.
80°C	The permeability of the cell membrane will increase excessively which lead to destroy the maintained balance of chemical concentrations within cells.
100°C	At this level water exist within tissues will be vaporized lead to large increase in volume and gas bubbles formation which induce mechanical ruptures and thermal decomposition of tissue fragments
>150°C	Carbonization which is the blackening of the nearby tissue, smoking and charring.
>300°C	Tissues will undergo melting when the temperature level elevated to few hundred degrees Celsius

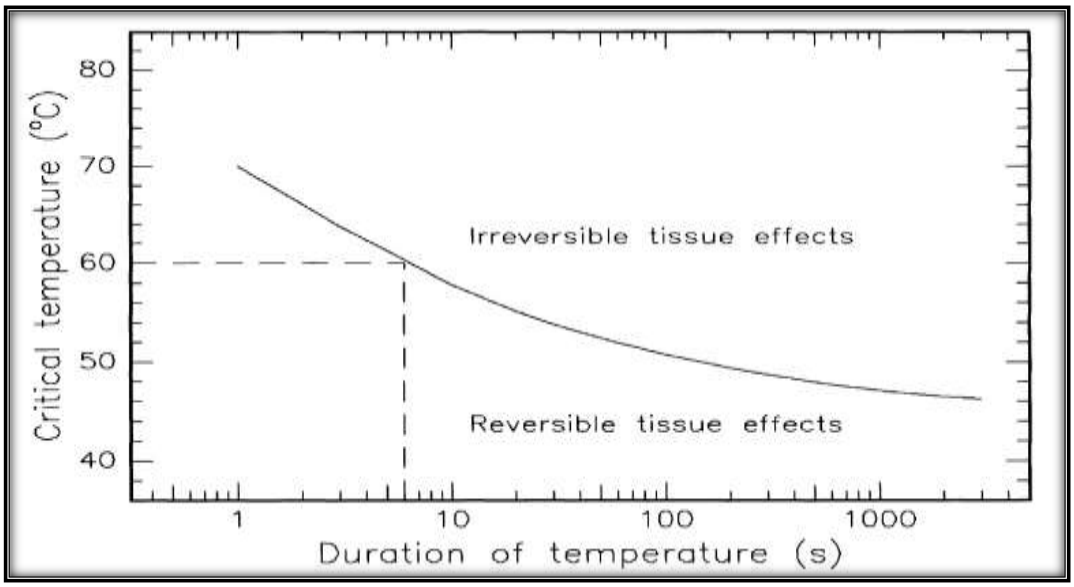

Figure 1-8 Relation between temperature duration and minimal critical temperature (Niemz, 2013).

The heat induction in the tissue depend on two factors:

1. Laser parameter like wavelength, spot size, exposure time, intensity of laser.
2. Tissue properties as coefficient of absorption, coefficient of scattering, thermal relaxation time, penetration depth.

The transmission of heat is by conduction, radiation and convection by blood flow, this transmission depend on thermal tissue characteristics like capacity and conductivity. The thermal influence on tissue is depends on two factors which are the type of tissue and temperature volume achieved inside the tissue. According to temperature duration various effects can occurs as shown in figure (1-9). (Niemz, 2013).

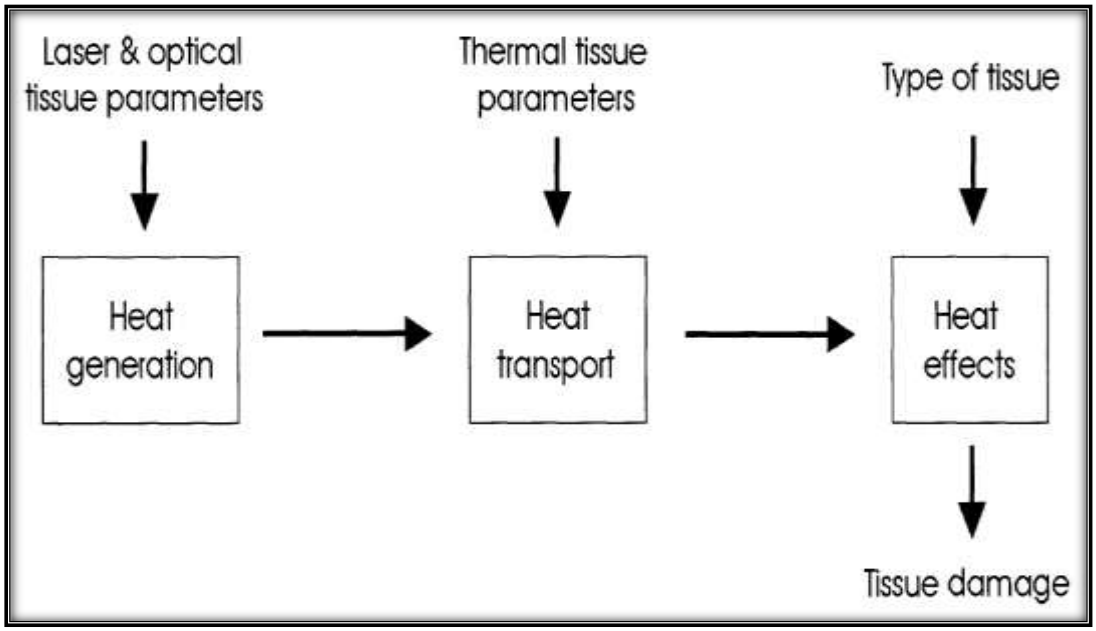

Figure 1-9 Flow chart for thermal tissue interaction (Niemz, 2013).

There are two important factors to be considered during photothermal effect which are

1. Thermal relaxation time: T therm, is the time needed by tissue to loss 63% of its thermal energy. The relationship between duration of pulse and time of thermal relaxation, when the thermal relaxation time is more than pulse duration there is no heat diffusion and no thermal damage, but when tissue has insufficient time to dissipate the heat so heat diffusion occur which end with thermal damage (Convissar, 2015).

2. Penetration depth ztherm (t), it represent the distance in which temperature descend to 1/2 of its peak value. Table (1-3) shows the relation between time and penetration depth of water.

Table (1-3) Relation of time with water penetration depth (Niemz, 2013).

Time t	Thermal penetration depth $z_{\text{therm}}(t)$
1 µs	0.7 µm
10 µs	2.2 µm
100 µs	7 µm
1 ms	22 µm
10 ms	70 µm
100 ms	0.22 mm
1 s	0.7 mm

2. Photochemical interaction:

When the exposure time is within seconds to continuous (long exposure time) and the power densities are very low (typically 1W/cm^2) the photochemical interactions occur. Laser light interact with macromolecules in tissue which induce chemical effects and reactions. Photochemical interaction mechanisms represented by photodynamic therapy (PDT) and biostimulation (Niemz, 2004).

I. Photodynamic therapy (PDT)

The modern period of PDT started at the Mayo Clinic in 1960 with studies of Lipson and Schwartz, they noticed that injection of crude preparations of hematoporphyrin result in neoplastic lesions fluorescence during surgery which aid in lesion visualization and after that significant work done to maximize efficiency and to treat human tumor with best result (Colussi et al., 1996).

PDT is a comparatively recent treatment method for neoplastic and non-neoplastic diseases. It requires the presence of three important components which are photosensitizer, light source with specific wavelength and molecular

oxygen. Photosensitizers are the compounds that absorb the light energy and induce reaction in non-absorbing molecules (Elson and Foran, 2015).

The photosensitizers designed in such way to enhance the uptake selectively and utilize the benefit of deep penetration of longer wavelength light (Dougherty et al., 1998).

Mechanism of PDT

The initiation of PDT process is occur when the photon energy is absorbed by photosensitizer and affords sequential decay which result in intramolecular energy transfer reactions. Three reactions are reaction type I (photo-oxidation radicals), reaction type II (singlet oxygen photo-oxidation) and reaction type III (photoreaction not involving oxygen), (convisar 2015).

The activation of photosensitizer to the excited singlet state after light absorption. Decay back of molecules to the ground state result in light emission

(fluorescence), heat dissipation or they can cross to the triplet state (Elson 2015).

II. Biostimulation: The concept of biostimulation is via increase adenosine triphosphate production (ATP) which results in:

A. Increase the endorphin production which inhibits nociceptive signals and creating varicosities transmission along the neurons which will decrease impulse transmission, these two action lead to decrease the pain.

B. Increase the collagen production and enhance microcirculation which improves the repair of tissue.

C. Increase the permeability of vessels wall and lymphatic circulation, decreasing tumor necrosis factor (TNF) production and proinflammatory interleukin which enhance the systemic effect of immune cells like natural killer cells and macrophage (Mester, 2013).

3. Photoablation:

Its principle is the braking of the organic molecular bonds in collagen and protein, so the photon energy should be higher than the dissociation energy of the bonds usually between 3 – 7 eV and the wavelength is under 350 nm. Usually this occurs with power density between 10^7 and 10^{10} W/cm^2 and short pulse duration (nanosecond), (Steiner, 2002). Figure (1-10) Scheme for principles of photoablation. This ablation technique provides precise etching process without thermal damage to the adjacent tissues, therefore tissue removal with this technique is highly predictable (Niemz, 2004).

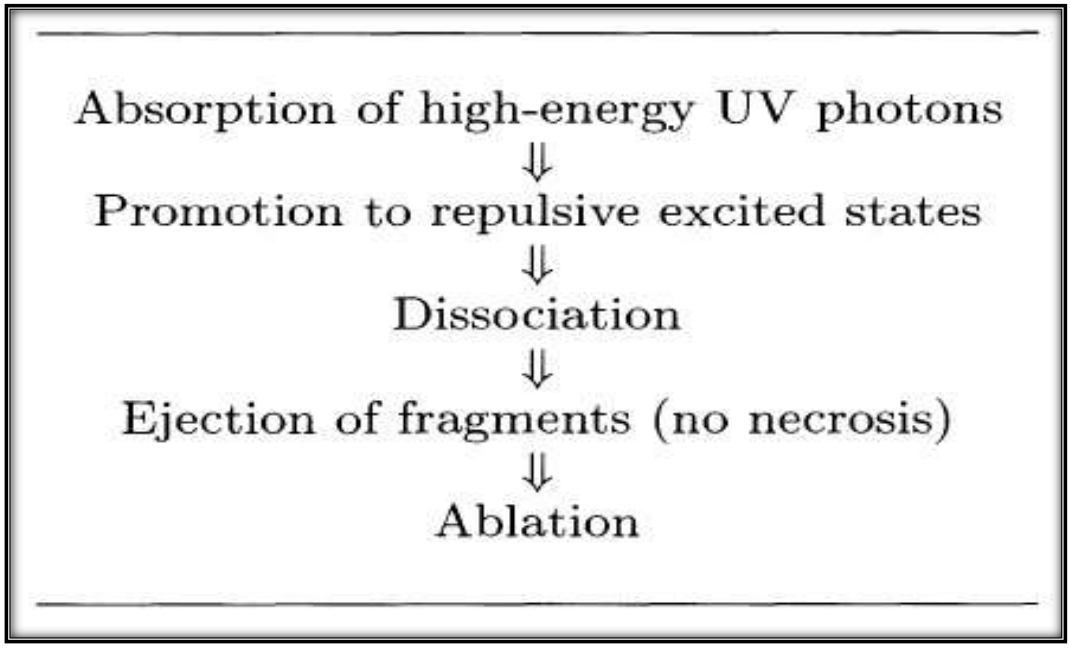

Figure 1-10 Scheme for principles of photoablation (Niemz, 2013).

1.11.2. Wavelength independent mechanism

1. **Plasma-induces photoablation**

When power density is above 10^{11} W/cm^2 with short pulse duration an optical breakdown phenomenon occur and accompanied with plasma formation and sparking noise. When electric fields are strong enough to strip electrons from their atoms lead to medium ionization and plasma formation (Steiner 2002).

2. Photodisruption:

High optical breakdown occurs when pulse duration is in picosecond or nanosecond, result in photodisruption. There is a popping sound which indicate chemical reaction occurrence and is accompanied to optical break down during plasma formation. (Lin et al., 2010).

1.12. Laser hazards

1.12.1. Classification of laser hazard (Smalley, 2011).

Class 1 laser system

These laser systems not lead to eye or skin injury during normal operation.

Class 1M laser system

These laser system not causing any hazard but should not be viewed by a collecting optics.

Class 2 laser system

These laser are visible in low power not lead to eye damage because of aversion response of human eye (blinking of the eye), but if viewed directly for period of time could cause potential eye damage.

Class 2M laser system

Visible lasers with low power not causing eye damage unless viewed by a collecting optics.

Class 3R laser system

These laser systems if viewed directly for a short period can produce an injury to the human's eye and can cause a greater eye hazard if it is viewed by a collecting optics.

Class 3B laser system

These lasers with medium power which are visible or invisible are able to produce a potential eye injury if viewed directly and specular reflection conditions. Diffuse reflection is not causing any eye injury.

Class 4 laser system

The high-power Visible or invisible lasers which is able to induce skin and eye injury if viewed directly as well as specular and diffuse reflection. These lasers are able of producing fire hazard also.

1.12.2. Types of Laser hazards

There are two types of laser hazards:

I. Laser beam hazards (SD Benjamin and J LeBeau, 2014).

A. Skin Burn: when operating high power lasers, all people inside the laser room must be protected from laser light. Dry fabrics, Alcohol containing solutions, foam devices, and rubbers which are flammable materials may ignite if exposed to laser and may cause skin burn, so source of water should be available in order to use it in case of igination.

B. Ocular damage: eye injury depends on the interaction of the light with the eye and the amount of the absorbed light. The type of the eye damage may be affected by laser parameters and the method of laser usage, so protective eye goggles according wavelength should wear by all the staff and the patient. Figure (1-11) Illustrate the potential eye damage according to laser wavelengths.

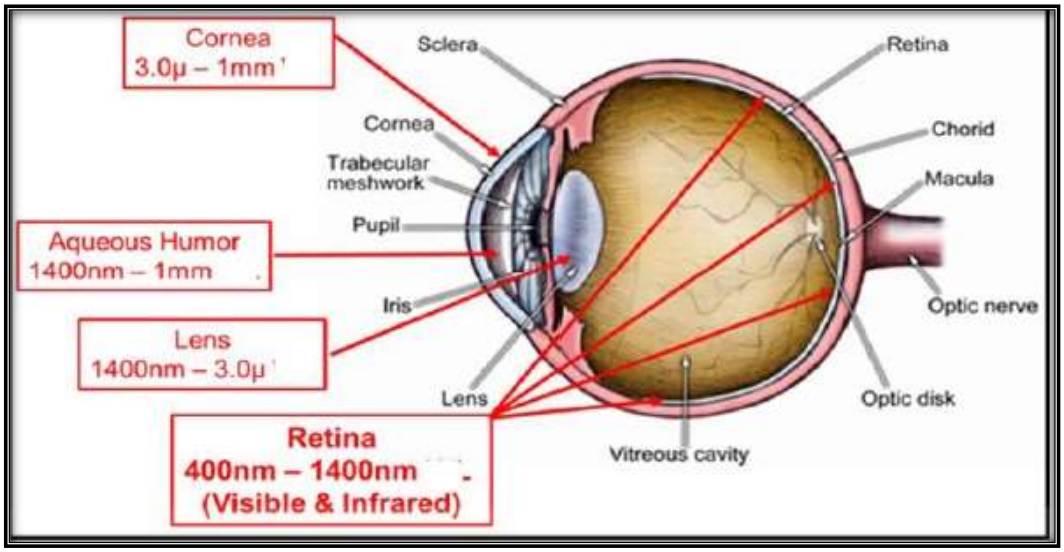

Figure (1-11) Potential eye damage according to laser wavelengths (SD Benjamin and J LeBeau, 2014).

II. Non laser beam hazards (Niemz, 2002).

A. Fire hazards

The fiery material like paper tray cover or dental gauze must be placed away from the hot fiber of the laser and any material such as oxygen, nitrous oxide and alcohol should be avoided.

B. Chemical hazards

The laser system which contain chemicals like Excimer, dye laser and chemical lasers which are dangerous and contains substances with highly toxicity that should be used with adequate precaution.

C. Electrical hazards

In the laser operation room the electrical cords and cables should be in suitable condition and so the electrical connection must be with proper grounding. Protective gloves should be warns when contact with electrical parts.

D. Respiratory hazards

Laser plume generation as a result of laser tissue interaction which is usually composed of fume of gases due to tissue ablation and also contain microorganisms and other hazardous particles. There must be a number of precautions to be considered to eliminate or decrease laser plume which include high filtration mask and high evacuation system.

1.12.3. Laser safety measures (SD Benjamin and J LeBeau, 2014).

I. Laser room (controlled area)

The area where one or more lasers are working and where personal activity must be controlled and supervised, these areas should:

1. Have physical dimensions for nominal ocular hazard zone.
2. Contain physical barrier walls, doors and windows in which all surfaces should be non-reflective and any laser beam should be attenuated.

3. Have high vacuum suction system to eliminate smoke plumes, the plume amount and other harmful matters produced is different according to the process, target tissue type, technique used and emission mode of laser.

4. Consist of suitable measures to assure that all laser supplier and system of delivery are preserved from an advertent damage.

6. Have a proper fire extinguisher should be sited with easy access

7. Have warning signs like:

A. "WARNING"

Indicates hazardous conditions cause serious trauma or death, which are these limited to class 3B and class 4 lasers.

B. "NOTICE"

These sings should include the classification of laser, wavelength used and output power, which indicates practice that does not cause any hazard.

C. "DANGER"

This sign is limited to class 4 laser with high output power which indicate an extensively hazardous conditions that lead to sever trauma or death if it's not avoided.

D. "CAUTION"

These limited to class 2 and 2M types of lasers, which indicates hazardous condition that can cause minor or moderate trauma.

II. Key measures for Staff and patient (Manson and Damrose, 2013).

1. Firing test to check all laser working components before starting the procedure clinically on the patient. The pigmented /dark articulating paper is the medium for visible and near infrared and for longer wavelength (mid- and far-infrared) the medium should be water.

2. Skin protection although is not consider as primary hazard for dentist opertaing with oral cavity, care should be taken with ablative procedure of vermilion border of lips to prevent encroaching beyond the target tissue.

3. Eye protection for all persons inside the controlled area for all class IV laser and other laser classes advised by individual manufacturers, the glasses/goggles should cover entire periorbital region, contains no scratches or damages, constructed for wavelength specific material and it should be marked if it is protect from specific wavelength or for band of wavelengths.

4. All the clinical staff within the clinical procedure should wear high-filtration well-fitting efficiency face masks, (0.1 micrometer in size) through whole procedure, standard surgical face mask is not sufficient for particles filtration.

1.13. Laser in dentistry

The lasers used in dentistry are two types hard tissue and soft tissue laser, depends on type of tissue chromophores and their absorption (Fleming M.G. and Maillet, 1999). Different types of laser used in dentistry shown in table (1-4).

Table 1-4 Types of laser used in dentistry (Sulieman, 2005).

Laser Wavelength nm	Active medium	Target chromophore	Uses in dentistry	Benefits	Drawbacks
Argon (488 – 514) nm	Gas (Argon gas)	Melanin & haemoglobin	Composite filling curing, Teeth bleaching, Soft tissue surgeries	Excellent haemostasis, Change surface chemistry of enamel & root dentin which reduce recurrent caries	Possibility of temperature elevation of pulp & adjacent tissue
Diode (810 , 830 , 940 , 980) nm	Semiconductor solids (GaAs)	Red pigments (Melanin & haemoglobin)	Power bleaching, periodontics, surgical soft tissue procedure	Good haemostatic ability	Poorly absorbed by water and the hydroxyapatite
Carbon dioxide (10600 nm)	Gas (Co2)	Water	Major and minor surgical soft tissue procedure	Rapid and excellent hemostasis with shallow penetration depth, high affinity for water thus rapid removal of soft tissue,	High cost and large size, Greater destruction of hard tissues due high absorbance.

Er,Cr:YSGG 2780 nm Er:YAG 2940 nm	Solid state **erbium**-doped yttrium aluminium garnet	Water & hydroxyapatite High water absorption rate	All tissue laser (hard & soft tissue procedure	Highest absorption in water & high affinity for hydroxyapatite	Poor haemostasis in soft tissue, time consuming, High cost
Nd:YAG 1064 nm	Solid state neodymium-doped yttrium aluminum garnet	Pigment (melanin and haemoglobin	Periodontics, Endodontics, surgical soft tissue procedure, bleaching	Effective in cutting and coagulation of oral soft tissues due to high absorption in pigments, Good hemostasis	Large size and high cost

The chromophores are light absorbing substances present inside tissues. Absorption of the laser light is related to wavelength and the chromophore. Most organic molecules show strong absorption in the ultraviolet (UV) region; therefore, the penetration depth in the UV region is very weak. In the visible region, absorption is mainly due to melanin and hemoglobin. Mid-infred and near-infrared region (600-1200 nm) the penetration depth is very high because these wavelengths are poorly absorbed. In Far-infrared region, the light is highly absorbed by water, and the penetration depth is shallow (Meesters, et al 2013). Figure (1-12) shows Different tissue chromophores and their absorption coefficient of laser wavelengths.

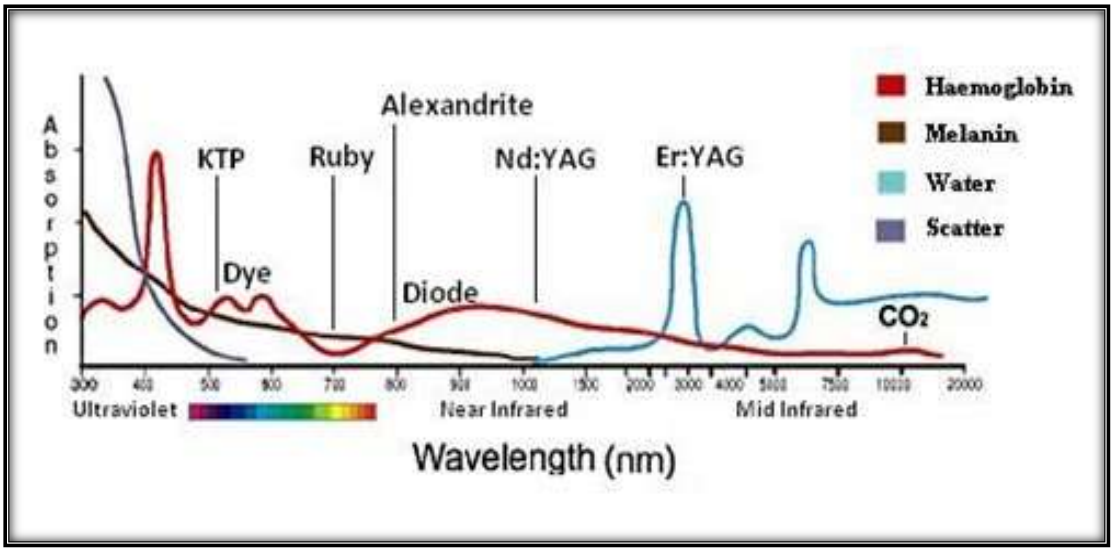

Figure 1-12 Different tissue chromophores and their absorption coefficient of laser wavelengths (Pirnat, 2007).

1.14. Laser in oral and maxillofacial region

The lasers have becoming the standard tool for many oral and maxillofacial procedures as with time are played entire role in maxillofacial surgery than other specialty. With laser a lot of surgical procedure can be done more successfully and efficiently than with electrocautry and scalpel. By utilizing the laser specific properties, many of new methods have been developed to done only by laser, so many of OMF surgeons shifting their work to laser surgery (Stabholz et al., 2003).

1.14.1. Advantages and disadvantages of laser in oral and maxillofacial surgery (Müller et al., 2006).

I. Advantages

1. An excellent vision of surgical site
2. Precise tissue cutting
3. Minimal post- surgical edema and swelling
4. Good hemostasis
5. Decrease scar and shrinkage of tissue
6. Bacterial elimination
7. Less instrument need in the surgical site .

II. Disadvantages:

1. High cost of laser device
2. Need special training
3. The overheating of tissue which must be avoided to prevent thermal tissue damage.

1.15. Diode laser application in surgical oral soft tissue procedures

The diode lasers used in medicine with wavelengths from 800 to 1064 nm, the active medium is gallium and arsenide (GaAs) which is a semi-conducting crystal (Manni, 2004).

Diode laser is the most favorable choice for oral soft tissue lesion excision, which seals the small blood vessels spontaneously and results in better vision, reduce cellular damage and improve healing. The controlled parameters by clinician are exposure time, power and spot size while others which mark the depth of effected zone of laser are uncontrollable factors. There are no constant parameters for all tissue procedures, for each case its own specific parameters to minimize the thermal tissue damage and get the best result (Strauss, 2000).

The surgeon should examine working site for wanted depth and decide if more passes through the tissues are needed. The laser wound is of different behavior form these of scalpel, there will be minimal adjacent tissue damage and formation of denaturation protein coagulum on the surface, so the pathologist should be informed about the laser that used for the procedure (Shokrollahi et al., 2004).

The Applications of diode laser in oral soft tissue surgical removal are:

A. In relation to oral soft tissue lesions (Clayman, and Kuo, 1997).

1. Mucoceles
2. Pyogenic granuloma
3. Fibroma
4. Peripheral gaint cell granuloma

B. In relation to prosthodontic (Convissar and Gharemani, 1995).

1. Epulis fissuratum reduction
2. Hyperplastic tissue reduction/vestibuloplasty
3. Soft tissue tuberosity reduction
4. Papillary hyperplasia
5. Denture stomatitis

C. In relation to periodontics (Neill, 1997).

1. Tongue tie
2. Frenoctomy

3. Gingivectomy

D. In relation to orthodontics (Sarver and Yanosky 2005).

1. Access to Unerupted Teeth

2. Excision of gingival hyperplasia due to improper oral hygiene

E. In relation to fixed prosthesis (Sarver and Yanosky, 2005).

1. Laser troughing

2. Crown lengthening

3. Ovate pontic design

4. Emergence profile

F. In relation to pedodontics (Kotlow, 2004).

1. Frenum Revisions

2. Impacted Mesiodens

3. Hyperplastic Gingival Tissue

1.16. External chromophores

1.16.1. Erythrosine stain

It is synthetic dye known as Red No 3 exist as red powder or granules, which is an organo-iodine compound. It is composed of 58% iodine (w/w), belongs to cyclic component known as xanthen and it has the ability to initiate photochemical reactions because its absorption is within visible and infra-red region of spectrum. It is manufactured via fluorescein iodination (Conlon and Berrios, 2001).

1.16.1.1 Chemical structure of erythrosine

The Erythrosine is soluble in water and slightly soluble in ethanol. It has band of absorption reach the near-infrared region with peak absorption at 530 mm (Conlon and Berrios, 2001). Figure (1-13) shows the chemical structure of erythrosine stain.

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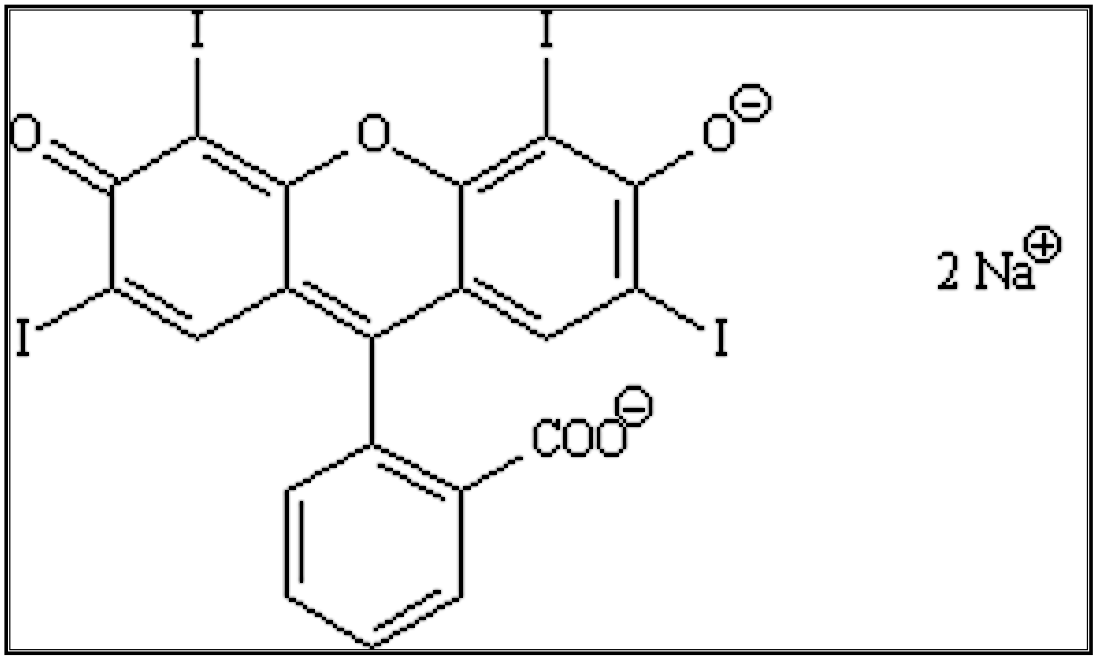

Figure 1-13 Chemical structure of erythrosine stain (Conlon and Berrios, 2001).

1.16.1.2. Uses of erythrosine

1. The erythrosine is highly applied to provide color for some drinks, cosmetics, pharmaceutical purposes because it provides a pleasing watermelon-red color when used in foods and pharmaceutical and it is used to give some tablet coating red color which may serve as iodine supplier in some food that may not be counted occasionally (Conlon and Berrios, 2001).

2. In dentistry the erythrosine used as disclosing tablets, solutions or patches to determine the area of dental plaque on the teeth surfaces (Sreenivasan and Gaffar, 2002).

The activity of erythrosine in photosensitizing oral microbes is well confirmed and one of the advantages of erythrosine from other photosensitizers is targeting the dental plaque directly and is fully approved for oral use (Datta et al., 2017).

1.16.1.3. Health effect of erythrosine

Chronic ingestion of daily diet containing 4% of erythrosine may provoke formation of thyroid tumor in rats by chronic stimulation of the thyroid by TSH. A series of studies concluded that the erythrosine is non-genotoxic compond and the increase in the Rats tumors is caused by a non-genotoxic mechanism (Jennings et al., 1990).

1.16.2. Methylene blue stain

It is a dye and medicine known as methylthioinm chloride, it is used mainly to treat methemoglobinemia when levels of methemoglobin is higher than 30% or when symptoms are exist despite oxygen therapy (Adams et al., 2007). Methylene blue was prepared firstly by German chemist Heinrich Caro in 1876. It has been firstly described as completely synthetic drug applied in medicine (Coulibaly et al., 2009).

1.16.2.1. Chemical structure of methylene blue stain

Methylene blue is a phenothiazine formal derivative, exist as powder of dark green color which produced blue colored solution in water, with hydrated form contain 3 molecules of water per unit of methylene blue. Methylene blue has band of absorption with peak absorption near 670 nm (Cenens and Schoonheydt 1988).

1.16.2.2. Medical Uses of methylene blue stain

1. It considered safe and effective method for treatment of selected cases of resistance plaque psoriasis by combination of methylene blue with water, as it considered a photosensitizer for PDT (Salah et al., 2009).

2. Used by Paul Guttmann and Paul Ehrilch in 1891 in the treatment of malaria (Calderon et al., 2017).

3. Use as mouth wash and in treatment of periodontitis (Aka et al., 2015).

1.16.2.3. Side effects of methylene blue stain

The common side effects of methylene blue includes confusion, vomiting, headache, shortness of breath, and high blood pressure. Methylene blue can cause toxicity in high doses, but is a safe drug when it used in therapeutic doses (<2mg/kg), (Coulibaly et al., 2009).

1.17. Literatures review of using erythrosine and methylene blue stains in dentistry

In Brazil, **Scwingel et al., (2012)** treated the HIV-infected patients complaining of oral Candidiasis by the Antimicrobial Photodynamic Therapy using combination of low power laser wavelength 660 nm and methylene blue 450 g/mL PDT is possible alternate for HIV patients with compromised immunity with destroying ability of virulence factors and low cost.

An in vitro study by **Lee et al. (2012)**, Demonstrated that there is a significant decrease in formation of S. mutans biofilm in response to PDT therapy using erythrosine stain and halogen curing unit which are commonly used in dental practice, which can be verified for controlling biofilm creating carious lesion.

(Aka et al 2015). A Comparative study between the cytotoxicity effects of methylene blue 1% and chlorhexidine gluconate used as mouth wash on the human gingival fibroblasts cell-lines in vitro. The study demonstrated that chlorhexidine gluconate available commercially 0.2% concentration is much more cytotoxic effect than methylene blue to human gingival fibroblast

The study of **Datta (2017)** concluded that erythrosine disclosing solutions are preparation containing dye or other coloring agents are recommended for the identification of bacterial plaque that can be distinctly seen providing a valuable visual aid and help in the maintenance of good oral health.

Prasopchai et al. (2017). Studie about the use of erythrosine as loaded fiber patches for plaque disclosing, The fiber patches had been fabricated via

electrospinning process and were characterized using scanning electron microscope (SEM). The study concluded that erythrosine-loaded fiber patches exhibited excellent wetting and disintegration properties and prompt released the erythrosine form the patches after contacted with saliva could be exploited as a plaque-disclosing material

Jeevanandan and Ganesh (2018), Study of 30 child patient to disclose dental plaque with erythrosine and fluorescein. The study reported that fluorescein differentiates between soft tissues and the plaques more appropriately as it stains the plaque and the soft tissue with different colors, while erythrosine stained both the surrounding tissues and plaque with red color.

Suzanne Tanya Nethan (2018). For early diagnosis of oral potentially malignant disorders (OPMDs), study of 50 cases from both sex with clinically diagnosed OPMDs found that the Methylene Blue (MB) 1% dye is an efficient diagnostic tool for large scale (OPMDs) with technique accuracy of 70%. It is simple to use and fairly sensitive method for oral cancer screening program for high risk individual.

In vitro study of **Agrwal et al. (2018)** compared the effect of adding different stains including the erythrosine on the incisions made by diode laser 940 nm. The study concluded that the stain can result in regular incision margins and reduce the lateral thermal damage.

Javali et al. (2019), In King Khalid University, an in vitro study evaluating the antimicrobial effects of PDT using methylene blue (MB) as photosensitizer and with laser as light source. The study concluded that PDT is effective method for killing the bacteria.

1.18. Aim of study

In vitro evaluations of external chromophores (erythrosine stain 3%, methylene blue1%) applications before cutting oral soft tissue with diode laser (940, 810) nm by:

1. Assessment of incision time clinically.
2. Histological evaluations of incision considering:

A. Depth and width of incision.

B. Depth and width of thermal damage.

C. Regularity and quality of incision).

Materials and Methods

This chapter describes in details all the materials and equipment used in this study which carried out a period between Jan. 2020 to Dec. 2020 at the Institute of Laser for postgraduate studies / University of Baghdad and Al-Imamen Al-Kadmen Medical City / Department of Maxillofacial Surgery, Baghdad, Iraq. The study had two phases, pilot study and an in vitro study.

2.1. Materials and equipment

This includes all the materials, tools and devices that had been used in this study which are listed in the table (2-1), figure (2-1) shows erythrosine stain 3% within plastic container, figure (2-2) shows Methylene blue stain 1% within plastic container and other materials are shown in figure (2-3).

Table 2-1 list of materials

No.	Chemical materials	Origin and company
1	Erythrosine stain 3%	India
2	Ethanol Alcohol 96%	Iraq
3	Formalin 10 %	China
4	Methylene blue stain 1%	Germany
5	Normal saline 0.9 %	AHLCON parenterals, India

Table 2-2 list of equipment

No.	Equipment	Origin and company
1	Cotton	Kardelen, Turkey
2	Disposable syringe	Shengguang medical instrument, China
3	Face mask	China
4	Gauze	Iraq
5	Gloves	CMC, Spine.
6	Marker	China
7	Micro-applicator brush : yellow 1002	Zogear, China

8	Plastic ontainer	China
9	Ruler	China
10	Scalpel	Pakistan
11	Sport clock	Sportline model 560, China
12	Surgical blade no.15	Demotek, Greece
13	Tissue forceps	Tissue forceps
14	Tray Towel	Tray Towel
15	Tweezer	Pakistan

Table 2-3 list of devices

No.	Devices	Company and origin
1	Diode laser 810 nm	Quick lase, UK
2	Diode laser 940 nm	(epic X, Biolase, USA)
3	Microtome	Leica,Germany
4	Optical microscope	Humanscope, Germany
5	Spectrophotometer	SHIMADZU, Japan

Figure (2-1) Erythrosine stain 3% with plastic container.

Figure (2-2) Methylene blue Stain 1% with plastic container.

Figure (2-3) Materials and equipment: 1. Mask, 2.gloves, 3.tissue forceps, 4.tweezer, 5.ruler, 6.marker,7. Surgical blade, 8.guaze, 9.disposable syringe, 10microapplicator, 11.normal saline, 12.sterile container, 13.cotton, 14.formaline 10%, 15.alcohol.

2.2. Devices

1. Diode laser 940 nm with maximum 10W power (Epic X, Biolase, USA), figures (2-4, 2-5).

Laser system specification according to the manufacturing manual:

- Laser class IV
- Wavelength: 940 ±10 nm
- Power accuracy: ± 20%
- Lithium ion battery
- Fiber optic delivery system.
- Medium: In GaAsP Semi-conductor diode .
- Surgical tips (200, 300 and 400 μm) .

- Maximum output power: 10W.
- Power mode: continuous and pulse 50 Hz .
- Tip initiation kit
- Wireless footswitch
- Surgical hand piece
- Screen console
- Aiming Beam: diode laser, max 1 mW, 625 nm – 670 nm, Class 2
- Protective eyewear

Figure (2-4) Diode laser 940 nm.

Figure (2-5) 1. Footswitch 2. Goggles 3. Initiation kit

2. Diode laser (810 + 980) nm (12w, dual plus 6, Quicklase, UK),

Figure (2-6)

Laser system specification according to manufacturing manual:

- Laser class IV
- Size w: 18.5 cm h:14.5 cm d:19 cm
- Weight 1.8 kg
- Medium: GaAIAs laser diode
- Wavelength: 810 and 980 +- 10 nm
- Output power: 0.1 – 12.0 Watts(+-)
- Input power Ac: 100 to 240 v, 1 amp 47-63 Hz
- Operation: continuous wave or pulsed at 10, 20, 50 Hz and adjustable up to 20000 Hz
- Fire cable 200-400 μm single file multi-mode
- Built in fibre optic caddy for fiber protection
- Fibre cable stripper
- Fibre cable ruby cleaver
- Disposable plastic tips
- 2 safety glasses with one for patient
- Foot pedal
- 6 color touch screen
- Laser category MD1104 GMDN 60340

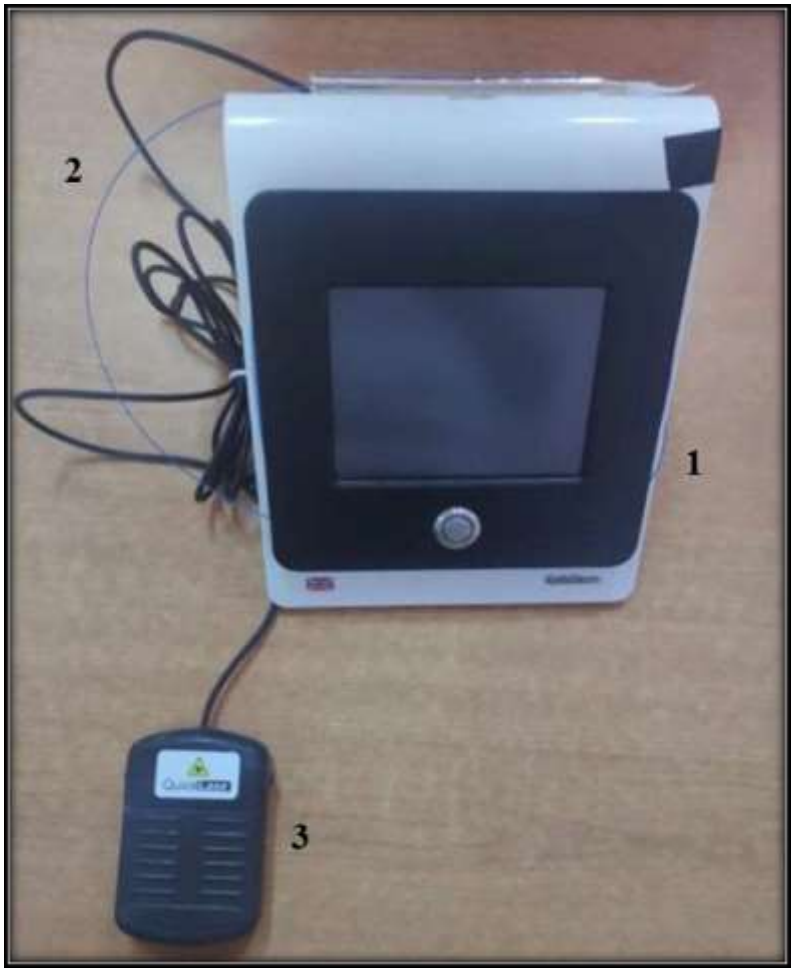

Figure (2-6) 1. Diode laser 810 nm, 2. Optical fiber, 3.footswitch.

3. Spectrophotometer SHIMADZU UV-1800 figure (2-7), (SHIMADZU CO. Malaysia).

Figure (2-7) SHIMADZU spectrophotometer

2.3. Methods

Mention of different methods used for determination the spectrum of stains, samples collection and preparation, the pilot study, the surgical procedures by lasers and samples preparation for histopathology.

2.3.1. Spectrum of stains

The spectrum of stains determined by spectrophotometer

2.3.1.1. Spectrum of erythrosine stain 3%

The spectrum of erythrosine stain shows peak absorption is at 530 nm shown in figure (2-8) with absorption band extend 960 nm shown in figure (2-9).

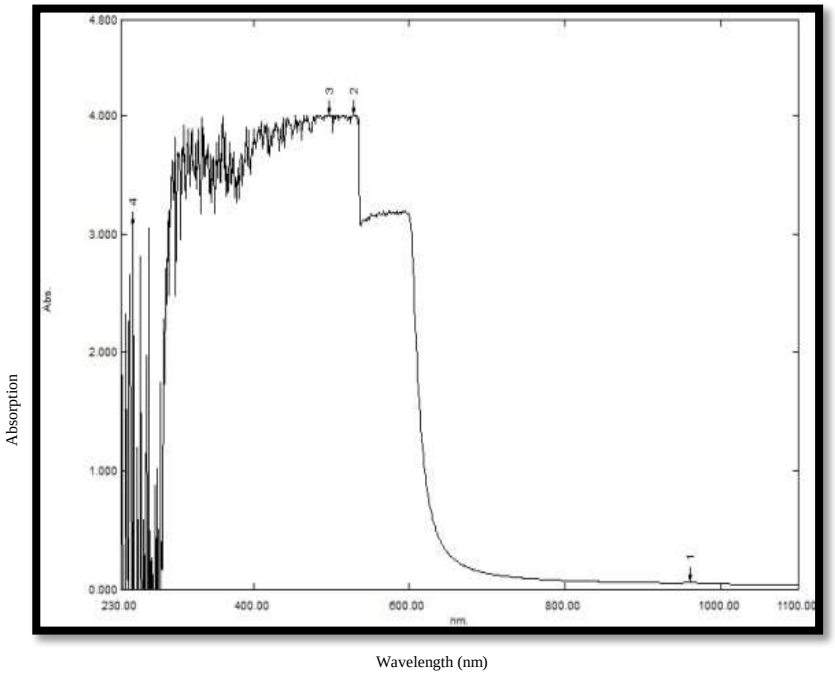

Figure (2-8) Absorption spectrum (0-4.8) of erythrosine stain 3%.

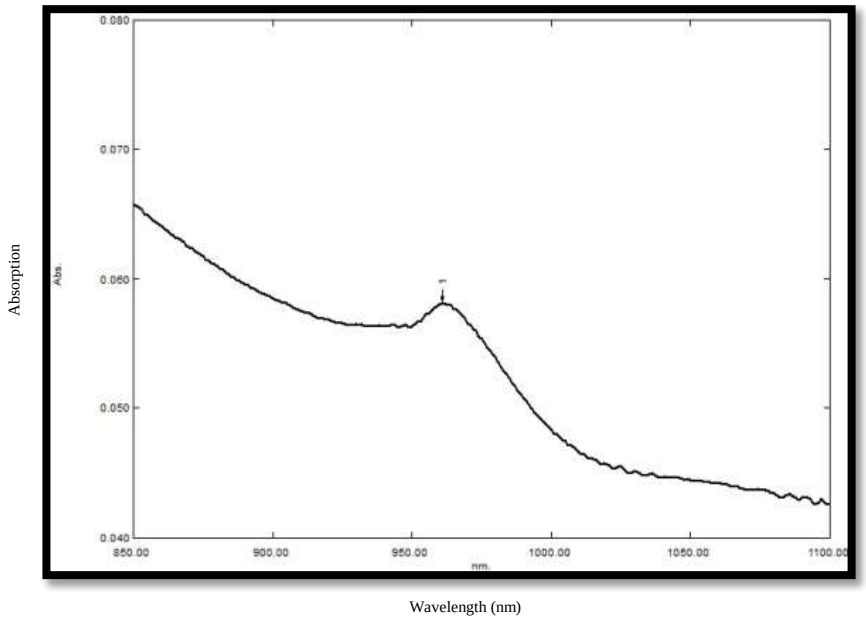

Figure (2-9) Absorption spectrum (0.04-0.08) of erythrosine stain 3%.

2.3.1.2. Spectrum of methylene blue stain 1%

The spectrum of methylene blue stain shows peak absorption is at 670 nm shown in figure (2-10) with absorption band extend to 900 nm shown in figure (2-11).

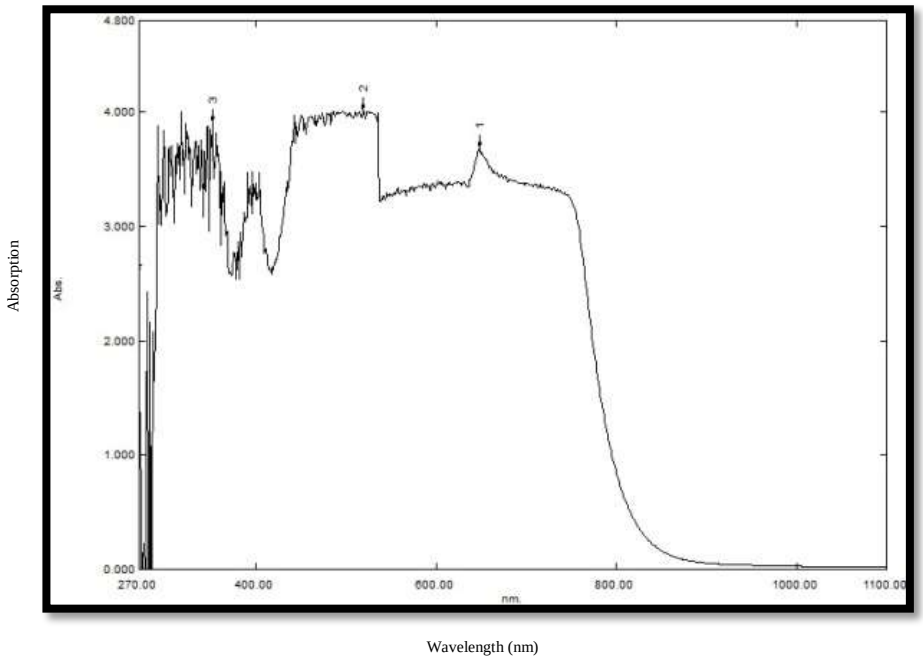

Figure (2-10) absorption spectrum (0-4.8) of methylene blue stain 1%

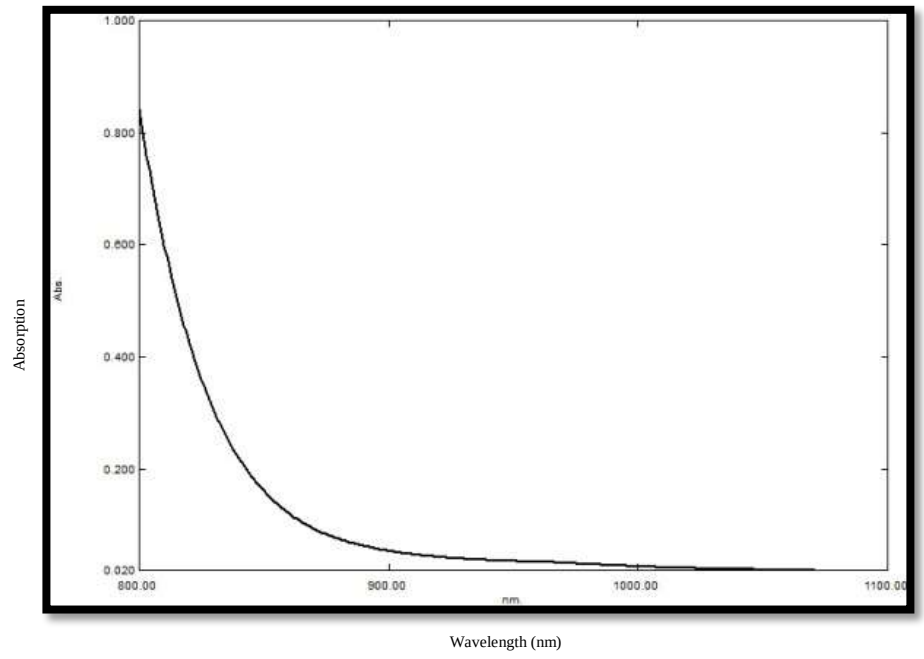

Figure (2-11) absorption spectrum (0.04 - 0.08) of methylene blue stain 1%.

2.3.2. Sample collection and preparation

Six fresh sheep tongues with dimensions about (8 cm length, 4 cm width, 2 cm thickness) collected directly after animal scarification cleaned by normal saline and divided by surgical blade no.15 into 60 samples with dimensions 1.5 cm length, 1cm width and with minimum of 0.5 cm thickness for erythrosine group and the same number for methylene blue group. Figure (2-12) shows sample ollection and preparation, figure (2-13) shows prepared samples with determined dimensions.

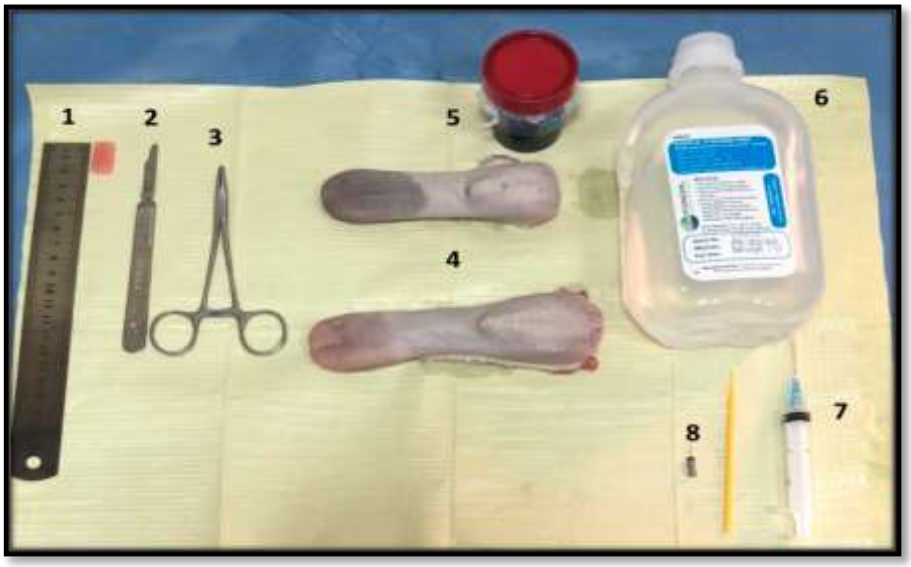

Figure (2-12) sample ollection and preparation: 1. Ruler 2. Scapel and blade 3. Tissue forcepes 4. Sheeps tongues 5. Stain 6. Normal saline 7. Disposable syringe 8. Microapplicator.

Figure (2-13) Prepared samples with determined dimensions.

2.3.3. Pilot study

In the pilot study 36 samples divided into three groups, 12 samples for each group, the incisions made with diode laser 940 nm, C.W, surgical initiated tip, table (2-3) shows the Pilot study grouping.

1st group: Power 1 W, 6 incisions without stain and 6 incisions with erythrosine stain 3%.

2nd: Power 2 W, 6 incisions without stain and 6 incisions with erythrosine stain 3%.

3rd: Power 3 W, 6 incisions without stain and 6 incisions with erythrosine stain 3%.

Table 2-2: Pilot study grouping

Wavelength	Power	Without stain	Sample no.	With stain	Sample no.
Diode 940 nm	1 W	Normal saline	6	Erythrosine 3%	6
	2 W	Normal saline	6	Erythrosine 3%	6
	3 W	Normal saline	6	Erythrosine 3%	6

The result of pilot study shows best incision time was for stained samples of power 3W group, the minimal thermal damage and best incision regularity was with power 2W group.

2.3.4. Samples Grouping

This study enrolled two groups. Each group contains 60 samples. All samples collected form fresh sheep tongues from local slaughter. Table (2-2) shows the Samples Grouping.

I. Group A: Diode laser 940 nm, power 2 W, C.W mode, initiated tip, 400 µm.

1. A I (control group), 20 samples without stain (normal saline).
2. AII (study group), 40 samples with erythrosine stain 3%.

II. Group B: Diode laser 810 nm, C.W mode, initiated tip

1. B I (control group), 10 samples without stain (normal saline).
2. B II (study group), 20 samples with methylene blue stain 1%. BI and BII with power 1.5W.
3. B III (control group), 10 samples without stain (normal saline).
4. B IV (study group), 20 samples with methylene blue stain 1%. B III and B IV power 2.5W.

Table 2-5: Samples grouping.

Group	Power	Without stain	Sample no.	With stain	Sample no.
Group A: Diode 940 nm	2 W	A I: Normal saline	20	A II: Erythrosine 3%	40
Group B: Diode 810 nm	1.5 W	B I: Normal saline	10	B II: Methylene blue 1%	20
	2.5 W	B III: Normal saline	10	B IV: Methylene blue 1%	20

2.3.5. Surgical procedures

I. Group A

The diode laser 940 nm used in C.W mode, power 2 W with surgical initiated tip 400 µm, the incisions made by directing the laser surgical tip perpendicularly on the dorsal surface of tongues and standardized incision length of 1cm and depth of 2 mm. For unstained samples incisions directly made and for stained samples incision made after application of erythrosine 3%

with micro-applicator brush along the planned incision line as shown in figures (2-14, 2-15).

Figure (2-14) sample stained with erythrosine stain 3%.

Figure (2-15) The planned incision with erythrosine stain 3%.

II. Group B

The diode laser 810 nm used in C.W mode, power (1.5, 2.5) W with surgical initiated tip with same surgical procedure as for group A except the stained samples with methylene blue 1%. as shown in figure (2-16).

Figure (2-16) The planned incision with methylene blue stain 1%

2.3.6. Samples preparation for histopathology

1. Fixation

Neutral buffered formalin (NBF) 10% was used for not less than 24 hours at 20-25 C°. The NBF prepared as: (Slaoui et al., 2011)

- Formaldehyde (37-40 %) Sharlau- Spain 100 ml
- Distal water (D.W) 900 ml
- NaH_2PO sodium di hydrogen phosphate 4.0 gm
- (Monobasic) Fluka-Germany
- Na2HPO4 sodium phosphate dibasic 6.5 gm
- (Anhydrous) Fluka-Germany

2. Dehydration

Tissues were transferred into ethyl alcohol as follows:

- 70 % ethyl alcohol for 2hrs
- 80% ethyl alcohol for 2hrs
- 90% ethyl alcohol for 2hrs
- Absolute alcohol (Scharlau-Spain) for 2hrs
- Absolute alcohol

3. Clearing

A-Xylene (UK) slides were immersed twice for 30 minutes

4. Embedding

The sections were put in an electrical wax dispenser contain molten paraffin wax (melting point range 58-60 C°) for 2 hrs., followed by second change which was lasted for another 2 hrs. in the embedding oven, then specimens were kept in refrigerator at 4 C° until they were used.

5. Sectioning

A serial 5 micrometers thickness section were obtained, using the rotary microtome (microtome, Germany), then sections were put into floating hot bath preheated to 40-45 C° release section from pressure.

For each case, four slides were taken, the first stained with Hematoxylin and Eosin to confirm the diagnosis and to determine the histological types and grades for the tumor, the second slide was put on the positively charged slide for immune histochemical staining with SOX2 antibody, third slide was stained with ALDH1A1 antibody, while the 4th slide was treated with EZH2 antibody.

6. De-waxing and hydration

The slides were transferred into an oven with temperature adjusted 60-65 C° for 5-10 min then transferred to xylene container for 20-30 minutes then sections were immersed in alcohol concentration bath 100%, 90% and 50% for one minute followed by washing the slides with distal water.

7. Staining and mounting

The harris modified hematoxylin was used for staining, the solution was prepared as below:

A. Materials

- Hematoxylins 5g
- Absolute ethyl alcohol 50 ml
- Potassium alum 100 g
- Distilled water 950 ml
- Mercuric oxide 2.5g
- Glacial acetic acid 40 ml

B. Method

Alum was dissolved in warm distal water in a flask. The hematoxylin was dissolved in 100% absolute alcohol and added to the alum then mixture was rapidly boiled with addition of mercuric oxide, then was rapidly cooled by cold water, completed by glacial acetic acid then filtered and stored (Meloan and Puchtler, 1987).

Eosin 1% (Fluka) was used as a counter stain to distinguish different cell types regarding the cytoplasm, matrix and connective tissue.

Eosin prepared as follows:

Eosin yellowish (Fluka) 10g

D.W 990 ml

The staining procedure was made as follows:

Hematoxylin was used for few minutes then washing of the slides thoroughly with tap water for few minutes and stained with eosin for not more than 3 minutes, washed again then dehydrated in 70%, 80%, 90% and 100% of grated alcohol for 1-3 minutes each then immersed in xylene for few minutes and mounted with DPX.

2.3.7. The evaluated parameters

1. The incisions time was recorded by apple phone stopwatch for all samples. As the criteria of (Abdul Jaleel and Mahmood 2014) for time measurement.

2. The histological evaluation included:

A. Depth and width of incision determined by two standardized points vertically for the depth and two for the width measured by ruler of microscope according to criteria of Vaderhobli et al. (2010).

B. Depth and width of thermal damage is determined by measuring the distance from edge of incision to the end the thermal damage (coagulation zone) according to criteria of Azevedo et al. (2016).

C. Regularity and quality of incisions according to regularity scale (0-4) according to criteria of Vescovi et al. (2010) , The epithelial changes in the core includes core, cytoplasmic and membrane modification and possible loss of intraepithelial and subepithelial adhesion, modification of connective tissue including charring and desiccation.

The scale is classified:

1. (0) represent the worst incisional quality score
2. (≥2) considered regular, when the incision margin appear mostly with a smooth linear border.
3. (<2) irregular , in the presence of a rough and uneven edge in most of the incision
4. (4) represents the highest quality of incisions.

2.3.8. Statistical analysis

The results of this study were statistically analyzed by Statistical Analysis System- SAS (2012) program, the analysis includes:

1. Descriptive analysis:

A. Means, standard error (SE).

B. Graphical presentation by bar chart.

2. Inferential analysis:

A. Student t-test to compare the significant difference between two variables.

B. Chi-square test to compare significant between percentages.

Results, Discussion and Conclusion

This chapter includes the results, discussion, conclusion and suggestions for the future work.

3.1. Results

The results consist of data and statistical analysis of the both group A (diode laser 940 nm with erythrosine stain 3%) and group B (diode laser 810 nm with methylene blue stain 1 %).

3.1.1. Results of group A

The result includes the incision time and histological evaluations for AI and AII.

I. Incision time

The incision time was recorded in seconds and the student t-test used to compare the result of AI and AII revealed a high significant difference between them with p-value < 0.01 as shown in table (3-1), figure (3-1).

Table 3-1: Comparison between AI and AII in incision time (s).

Group A	Mean ± SD of incision time (s)	t-test	p-value
AI	13.54 ± 0.24	0.170	0.0001 **
AII	12.92 ± 0.19		

(NS) Non significant, (*) significant, (**) highly significant

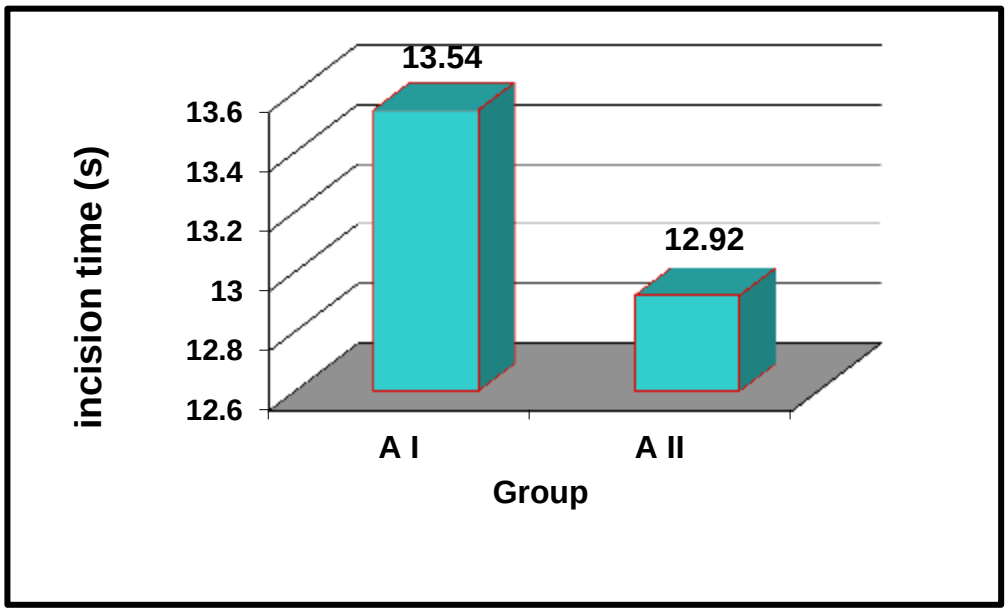

Figure 3-1 Bar chart of means incision time, AI = incision assisted with normal saline, AII= incision assisted with erythrosine 3%

II. Histological evaluations

Figure (3-2) illustrates the normal layers of mucosa as appeared histologically. The histological evaluations includes incision depth (I.D), incision width (I.W), damage depth (D.D), damage width (D.W) and incision quality and regularity as shown in figure (3-3) for AI and in figure (3-4) for AII.

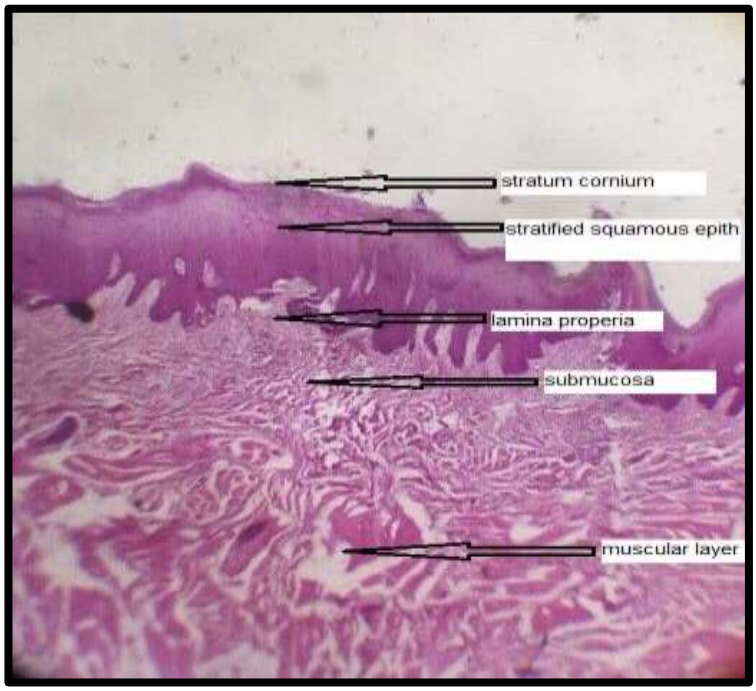

Figure (3-2) Microphotograph, illustrate normal histology of tongue, H&E, X 40.

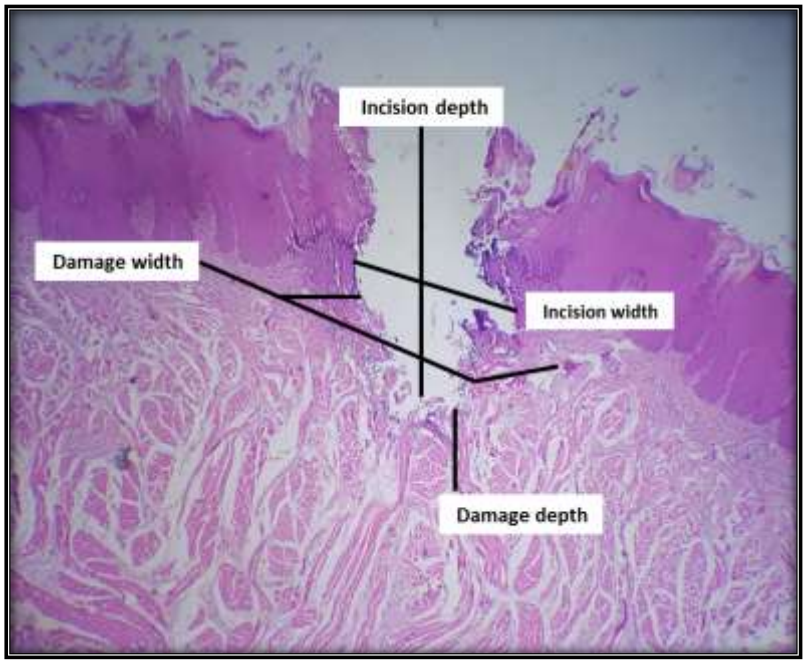

Figure (3-3) microphotograph, illustrates the histological evaluations (incision depth, incision width, damage depth, damage width) of sheep tongue of group AI, H&E, X 100.

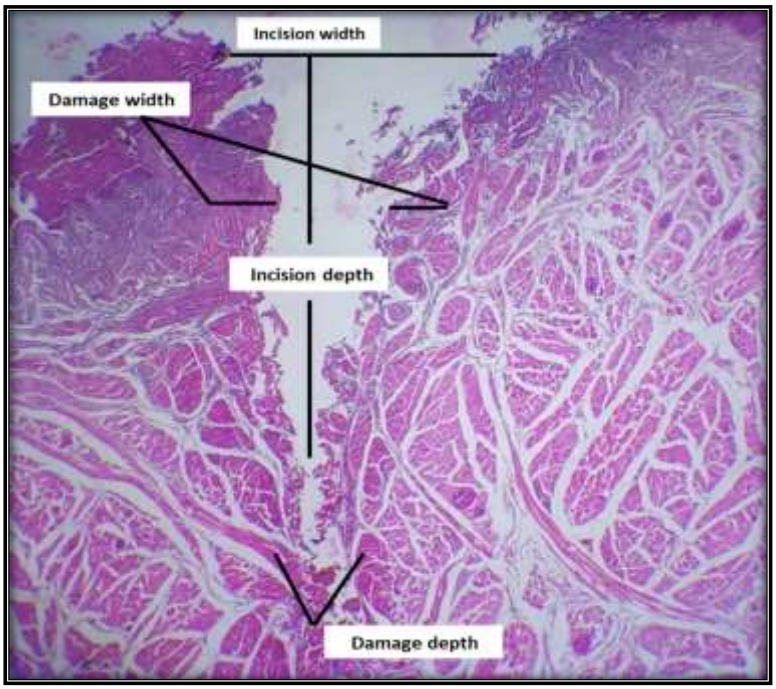

Figure (3-4) microphotograph, illustrates the illustrates the histological evaluations (incision depth, incision width, damage depth, damage width) of sheep tongue of group AII, H&E, X 100.

1. Incision depth

The results shows that there is no significant difference between AI and AII in the incision depth as shown in table (3-2), figure (3-5).

Table 3-2: Comparison between AI and AII in incision depth (mm)

Group A	Mean ± SD of Incision depth (mm)	t-test	p-value
AI	1.320 ± 0.66	0.330 NS	0.607 NS
AII	1.405 ± 0.75		

(NS) Non significant,(*) significant, (**) highly significant

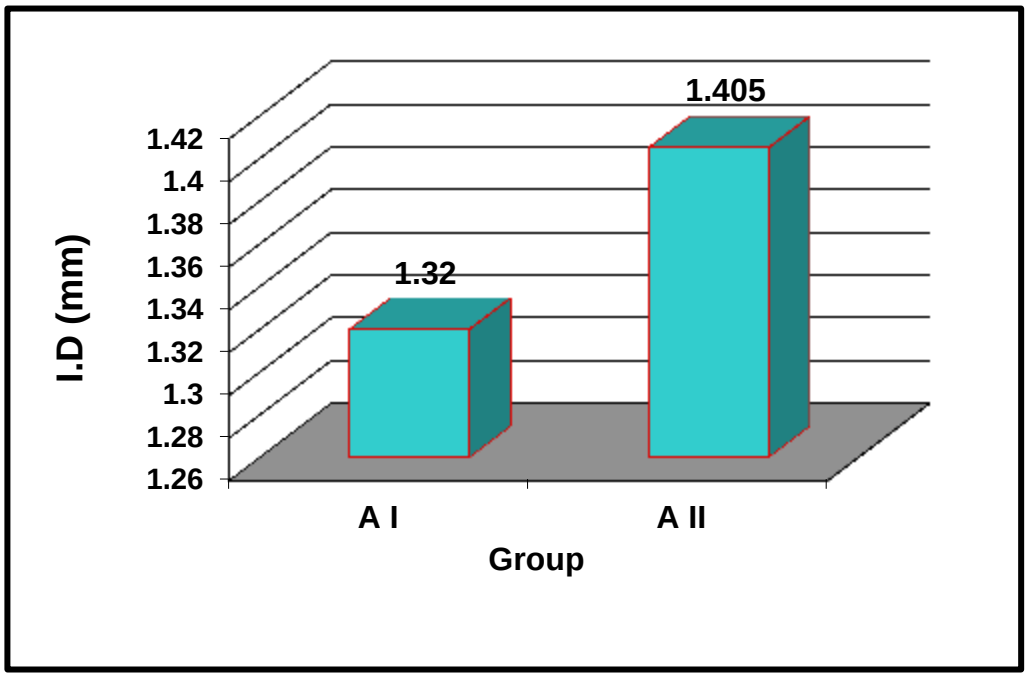

Figure 3-5 Bar chart of means incision depth, AI = incision assisted with normal saline, AII= incision assisted with erythrosine 3%

2. Incision width

There is no significant difference in the incision width between AI and AII as shown in table (3-3), figure (3-6).

Table 3-3: Comparison between AI and AII in incision width (mm).

Group A	Mean ± SD of Incision width (mm)	t-test	p-value
AI	0.465 ± 0.29	0.144	0.0884 NS
AII	0.589 ± 0.33		

(NS) Non significant,(*) significant, (**) highly significant

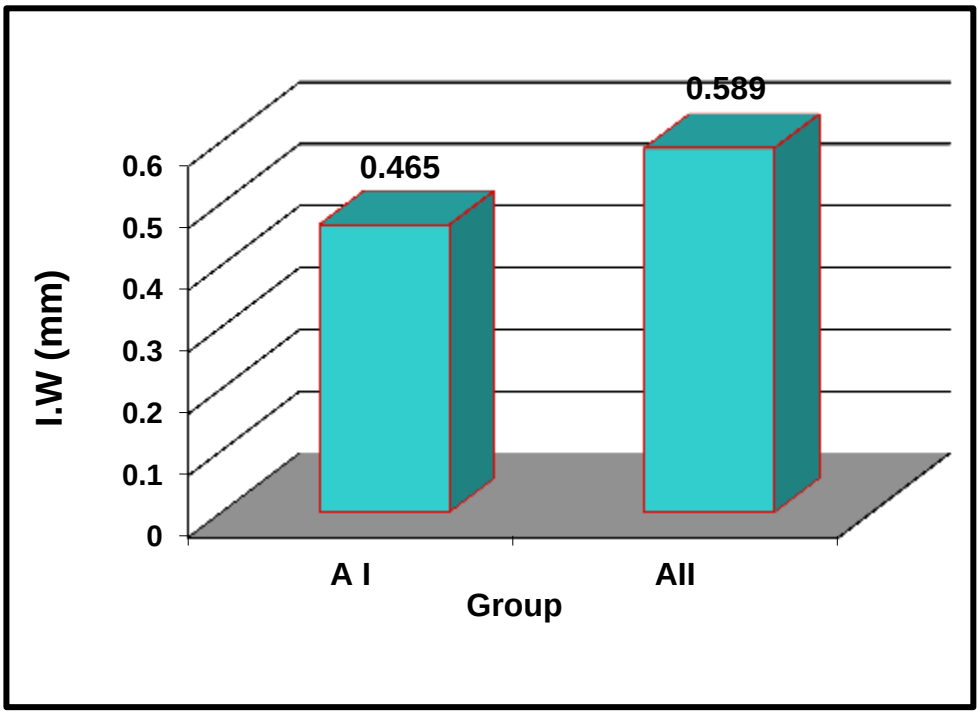

Figure 3-6 1 Bar chart of means incision width, AI = incision assisted with normal saline, AII= incision assisted with erythrosine 3%

3. Damage depth

There is a high significant difference between AI and AII in the damage depth with P<0.01 as shown in table (3-4), figure (3-7).

Table 3-4: Comparison between AI and AII in damage depth (mm).

Group A	Mean ± SD of damage depth	t-test	p-value
AI	1.105 ± 0.21	0.180	0.0002 **
AII	0.752 ± 0.18		

(NS) Non significant,(*) significant, (**) highly significant

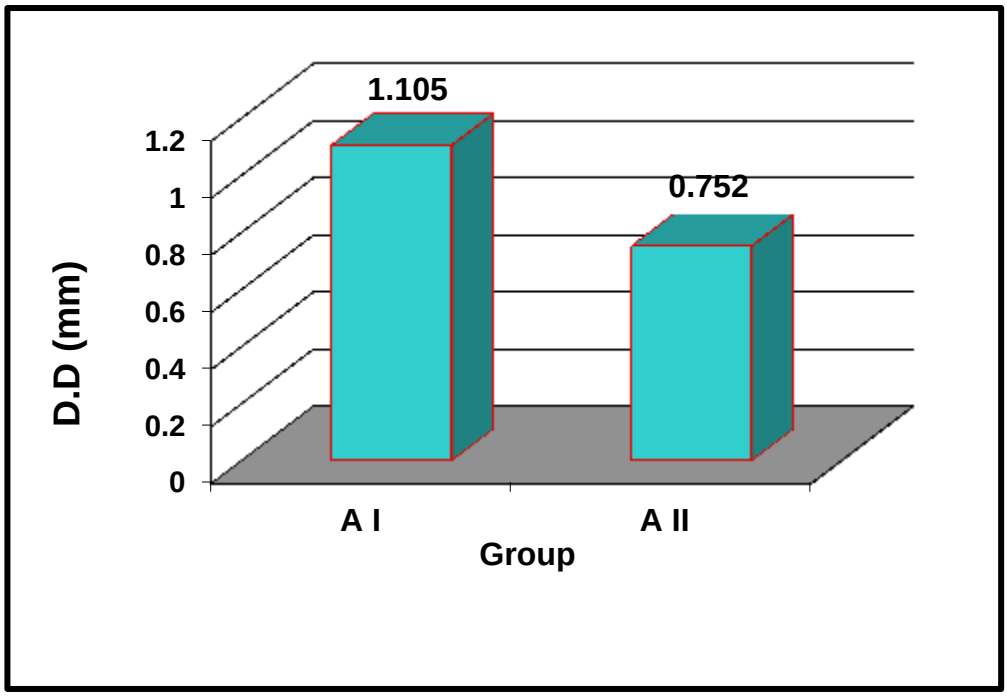

Figure 3-7 1 Bar chart of means damage depth, AI = incision assisted with normal saline, AII= incision assisted with erythrosine 3%.

4. Damage width

Results of data shows that variables are normally distributed among groups using Shapiro- Wilk test at p> 0.05, and there is a significant difference between AI and AII in the damage width with as shown in table (3-5), figure (3-8).

Table 3-5: Comparison between AI and AII in damage width (mm).

Group A	Mean ± SD of damage width	t-test	p-value
AI	0.770 ± 0.19	0.101	0.0025 **
AII	0.610 ± 0.16		

(NS) Non significant,(*) significant, (**) highly significant

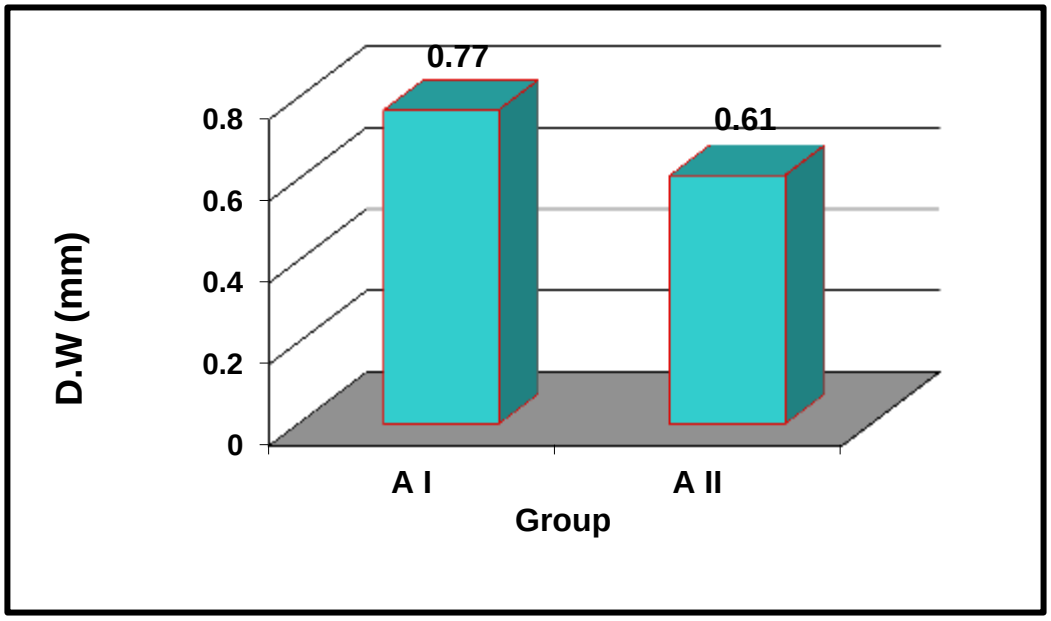

Figure 3-8 1 Bar chart of means damage width, AI = incision assisted with normal saline, AII= incision assisted with erythrosine 3%.

5. Regularity and quality scale

The result shows that that no significant difference in (0, ≥2) scale and there is high significant difference in (<2, 4) scale with p-value<0.01 between AI and AII, as shown in table (3-6).

Table 3-6. The distribution of samples according to Regularity and quality between AI and AII

Regularity and quality	AI: (%)	AII: (%)	P-value
0	0 (0.00%)	0 (0.00%)	NS
<2	9 (45.00%)	7 (17.50%)	0.0001 **
≥2	9 (45.00%)	16 (40.00%)	0.285 NS
4	2 (10.00%)	17 (42.50%)	0.0001 **
Total No.	20	40	--
** (P≤0.01)-Highly Sig , NS: Non-Significant			

3.1.2. Result of Group B

The result includes the comparison between BI and BII and between BIII and BIV in incision time and in histological evaluations.

1. Comparison the result of BI and BII and their statistical analysis.

I. Incision time

The result shows a high significant difference in incision time between BI and BII with P<0.01 as shown in table (3-7), figure (3-9).

Table 3-7: Comparison between BI and BII in incision time (s).

Group B	Mean ± SD of incision time (s)	t-test	p-value
BI	15.17 ± 0.22	0.1112	0.0001**
BII	14.79 ± 0.15		

(NS) Non significant,(*) significant, (**) highly significant

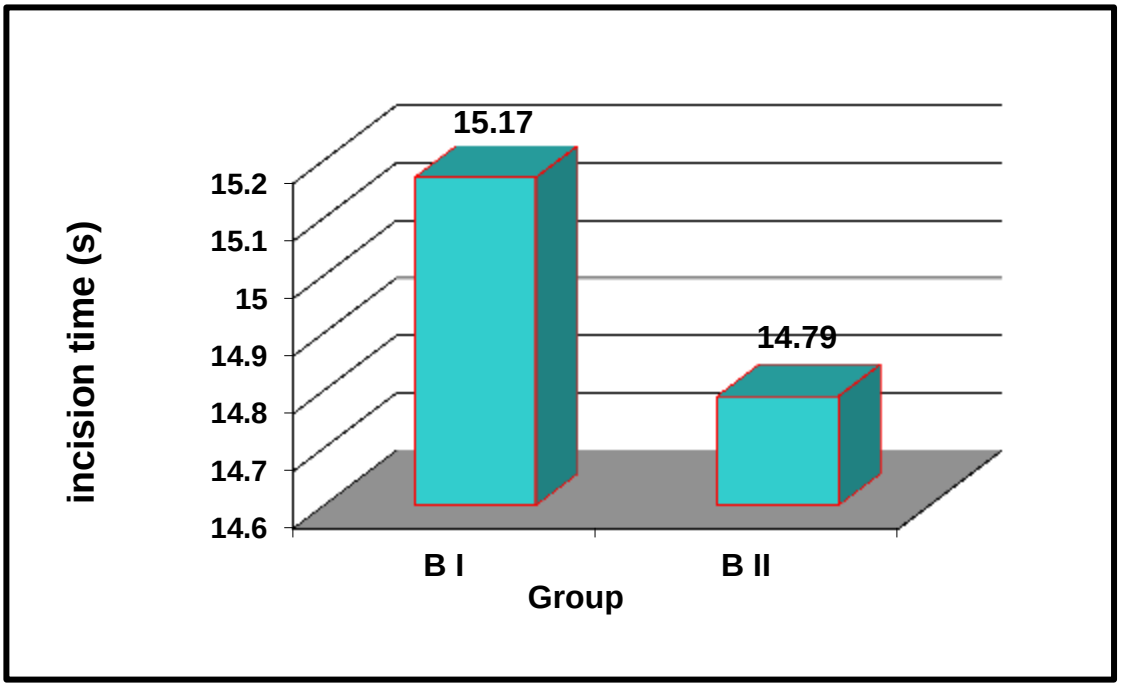

Figure 3-9 1 Bar chart of means incision time, BI = incision assisted with normal saline, BII= incision assisted with methylene blue 1%.

II. Histological evaluations

The histological evaluations includes comparison between BI and BII in incision depth (I.D), incision width (I.W), damage depth (D.D), damage width (D.W) and incision quality and regularity.

1. Incision depth

The result shows that there is a high significant difference between BI and BII in the incision depth with P<0.01 as shown in table (3-8), figure (3-10).

Table 3-8: Comparison between BI and BII in incision depth (mm).

Group B	Mean ± SD of incision depth (mm)	t-test	p-value
BI	1.71 ± 0.13	0.1059	0.0090**
BII	1.855 ± 0.18		

(NS) Non significant,(*) significant, (**) highly significant

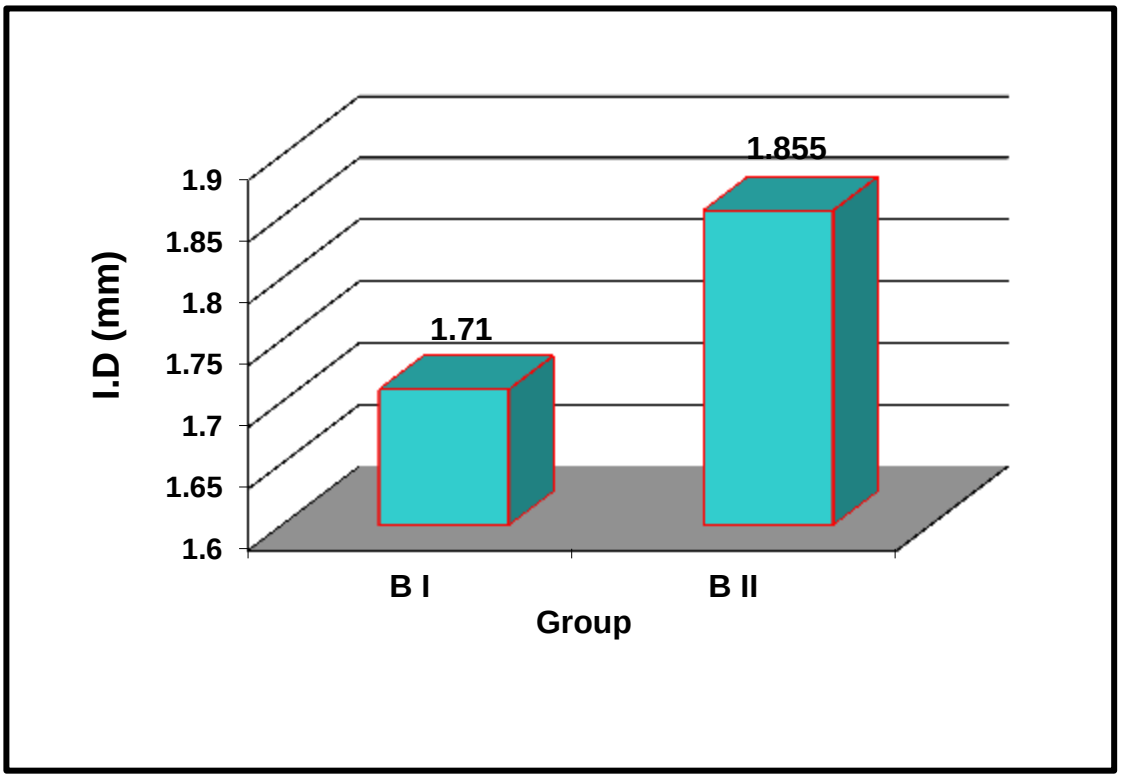

Figure 3-10 1 Bar chart of means incision depth, BI = incision assisted with normal saline, BII= incision assisted with methylene blue 1%.

2. Incision width

Results of data shows that variables are normally distributed among groups using Shapiro- Wilk test at p> 0.05, and there is a significant difference in the incision width between BI and BII as shown in table (3-9), figure (3-11)

Table 3-9: Comparison between BI and BII in incision width (mm).

Group B	Mean ± SD of incision width (mm)	t-test	p-value
BI	0.730 ± 0.16	0.1221	0.009**
BII	0.870 ± 0.11		

(NS) Non significant,(*) significant, (**) highly significant

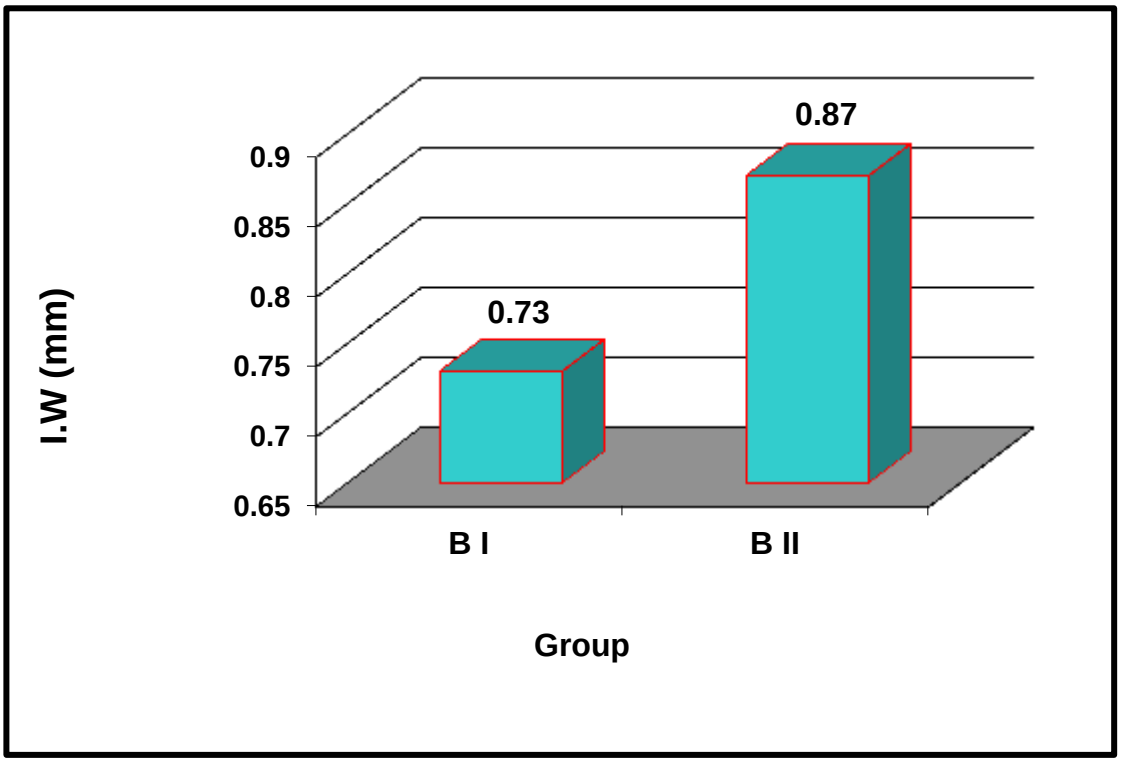

Figure 3-11 1 Bar chart of means incision width, BI = incision assisted with normal saline, BII= incision assisted with methylene blue 1%.

3. Damage depth

There is a significant difference between BI and BII in the damage depth with P<0.05 as shown in table (3-10), figure (3-12)

Table 3-10: Comparison between BI and BII in damage depth (mm).

Group B	Mean ± SD of damage depth (mm)	t-test	p-value
BI	1.19 ± 0.16	0.0922	0.044*
BII	1.095 ± 0.14		

(NS) Non significant, (*) significant, (**) highly significant

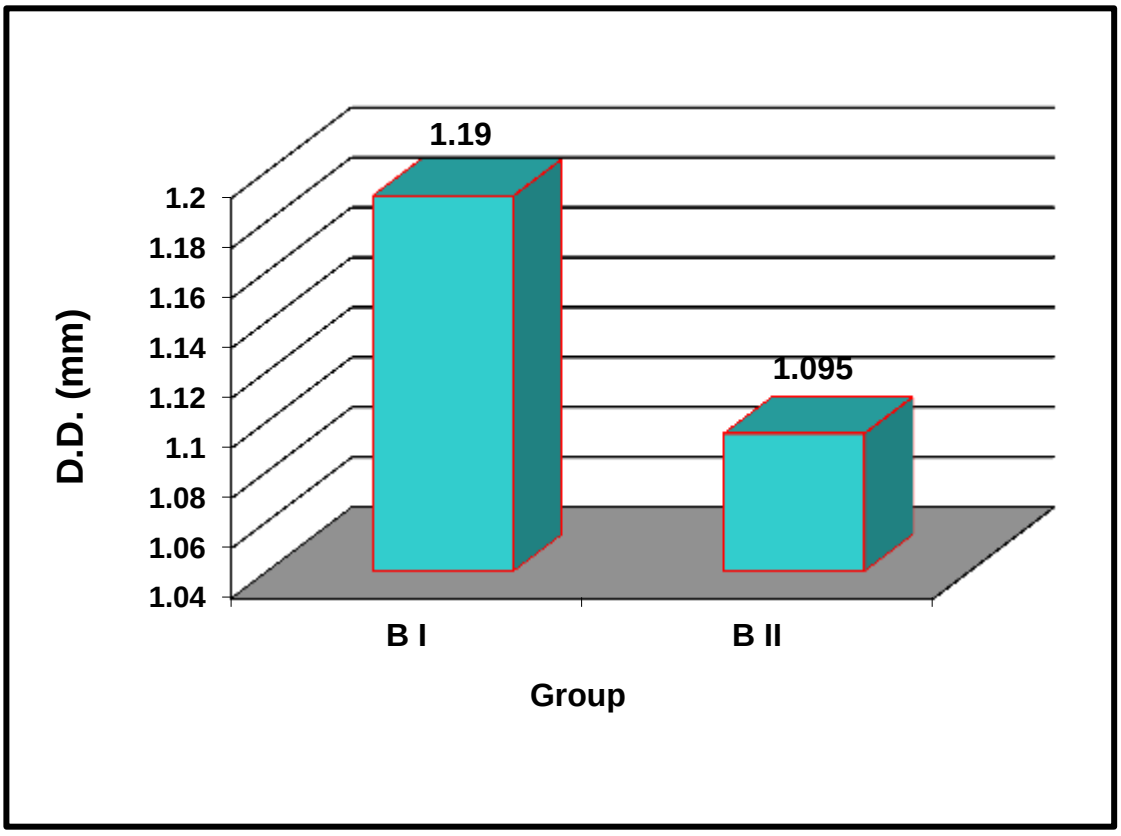

Figure 3-12 1 Bar chart of means damage depth, BI = incision assisted with normal saline, BII= incision assisted with methylene blue 1%.

4. Damage width

There is a high significant difference between BI and BII in the damage width with P<0.01 as shown in table (3-11), figure (3-13).

Table 3-11: Comparison between BI and BII in damage width (mm).

Group B	Mean ± SD of damage width (mm)	t-test	p-value
BI	0.980 ± 0.11	0.0906	0.0003**
BII	0.795 ± 0.14		

(NS) Non significant, (*) significant, (**) highly significant

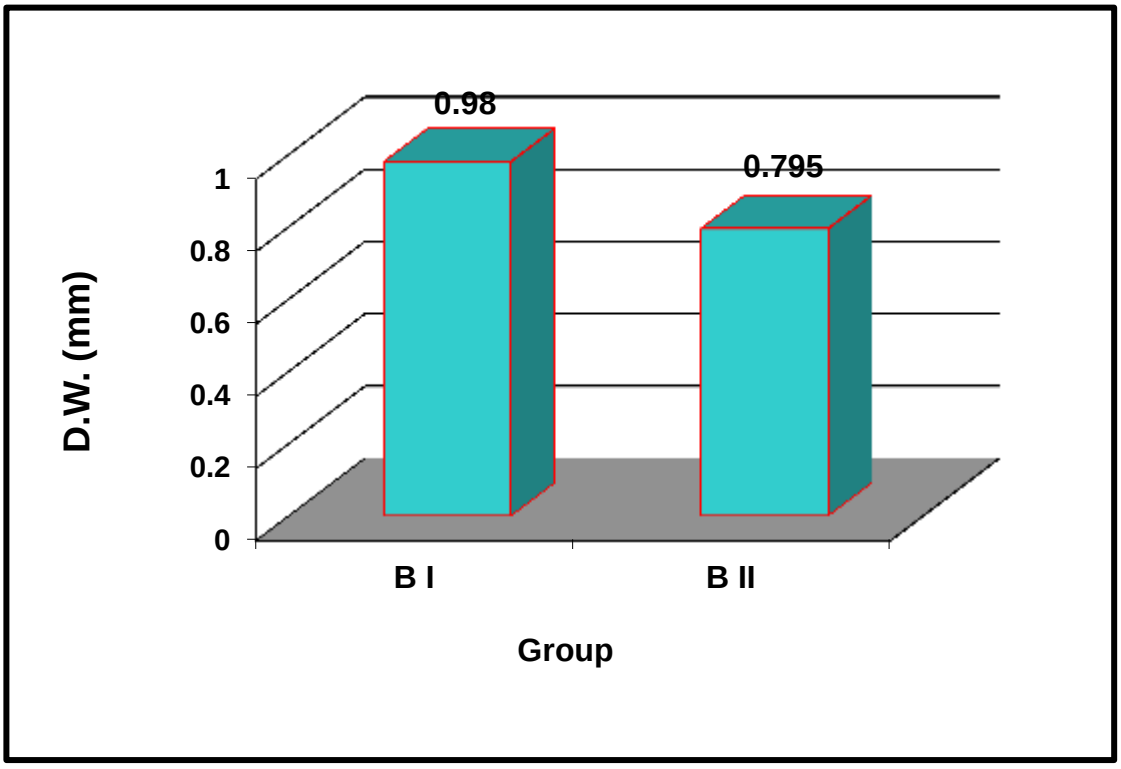

Figure 3-13 1 Bar chart of means damage width, BI = incision assisted with normal saline, BII= incision assisted with methylene blue 1%.

5. Regularity and quality scale

The result shows that that no significant difference in (0) scale and there is a significant difference in (≥2) scale with p-value<0.05 and in (<2, 4) scale with p-value<0.01 between BI and BII as shown in table (3-12).

Table 3-12 Distribution of sample according to Regularity and quality between BI and BII.

Regularity and quality	BI: (%)	BII: (%)	P-value
0	0 (0.00%)	0 (0.00%)	NS
<2	5 (50.00%)	3 (15.00%)	0.0001 **
≥2	5 (50.00%)	7 (35.00%)	0.0294 *
4	0 (0.00%)	10 (50.00%)	0.0001 **
Total No.	10	20	--
NS: Non-Significant , * (P≤0.05) - Sig , ** (P≤0.01)-Highly Sig.			

2. Comparison the result of BIII and BIV and their statistical analysis.

I. Incision time

The result shows a high significant difference in incision time between the BIII and BIV with p-value<0.01 as shown in table (3-13) and figure (3-14).

Table 3-13: Comparison between BIII and BIV in incision time (s).

Group B	Mean ± SD of incision time (s)	t-test	p-value
BIII	13.58 ± 0.26	0.196	0.0001**
BIV	12.97 ± 0.17		

(NS) Non significant, (*) significant, (**) highly significant

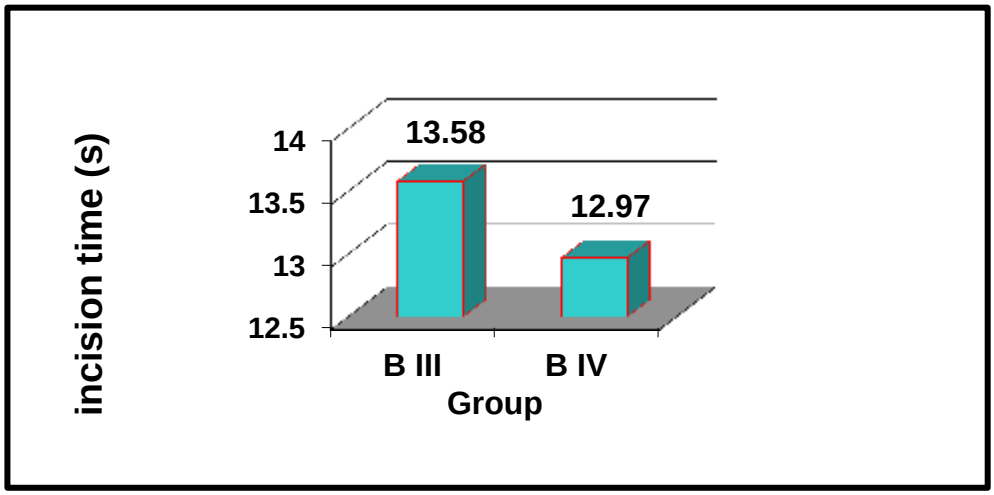

Figure 3-14 1 Bar chart of means incision time, BIII = incision assisted with normal saline, BIV= incision assisted with methylene blue 1%.

II. The histological evaluations includes comparison between BIII and BIV in incision depth (I.D), incision width (I.W), damage depth (D.D), damage width (D.W) and incision quality and regularity as shown in figure (3-15) for BIII and figure (3-16) for BIV.

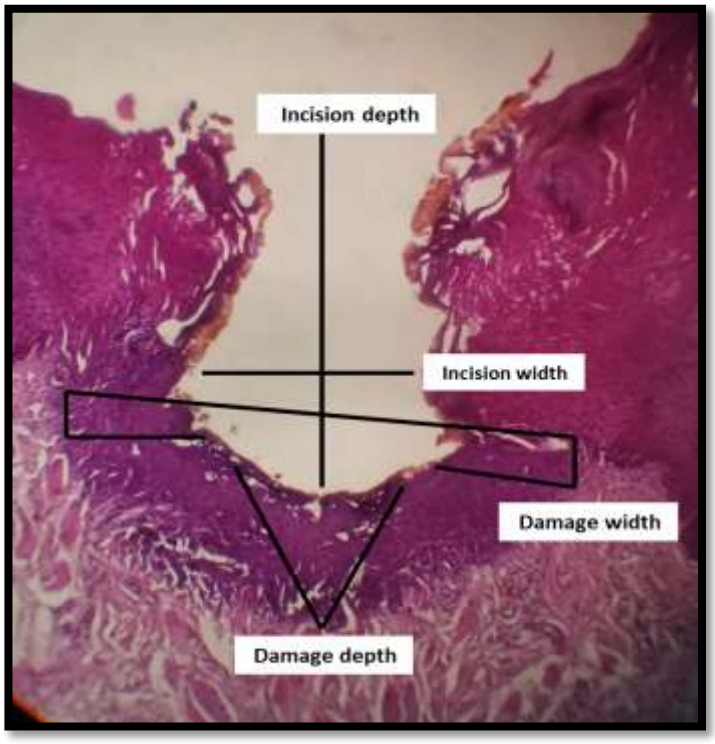

Figure (3-15) microphotograph, illustrates the illustrates the histological evaluations (incision depth, incision width, damage depth, damage width) of sheep tongue of group BIII, X 100.

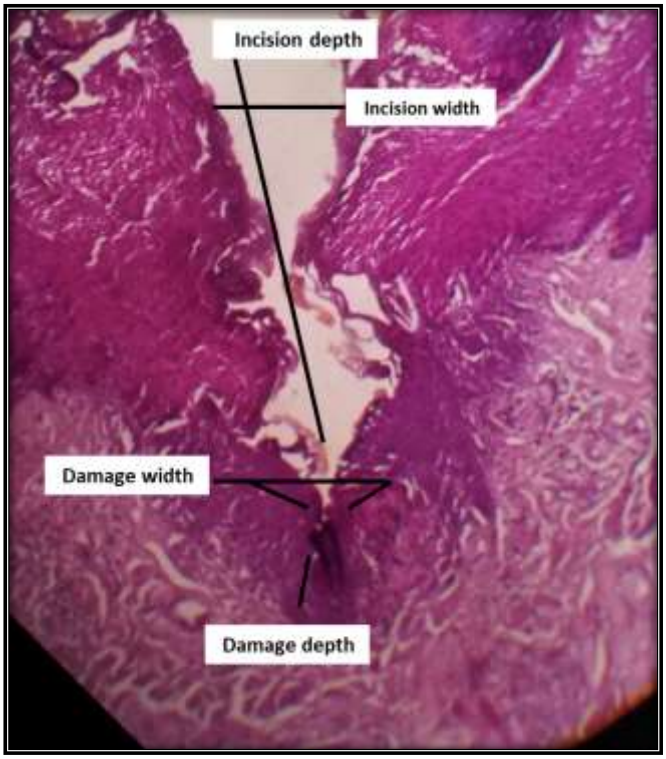

Figure (3-16) Microphotograph, illustrates the histological evaluations (incision depth, incision width, damage depth, damage width) of sheep tongue of group BIV, X 100.

1. Incision depth

The results shows that there is a high significant difference between B III and B IV in the incision depth with P<0.01 as shown in table (3-14), figure (3-17).

Table 3-14: Comparison between BIII and BIV in incision depth (mm).

Group B	Mean ± SD of incision depth (mm)	t-test	p-value
BIII	1.76 ± 0.62	0.130	0.0009**
BIV	1.99 ± 0.47		

(NS) Non significant, (*) significant, (**) highly significant

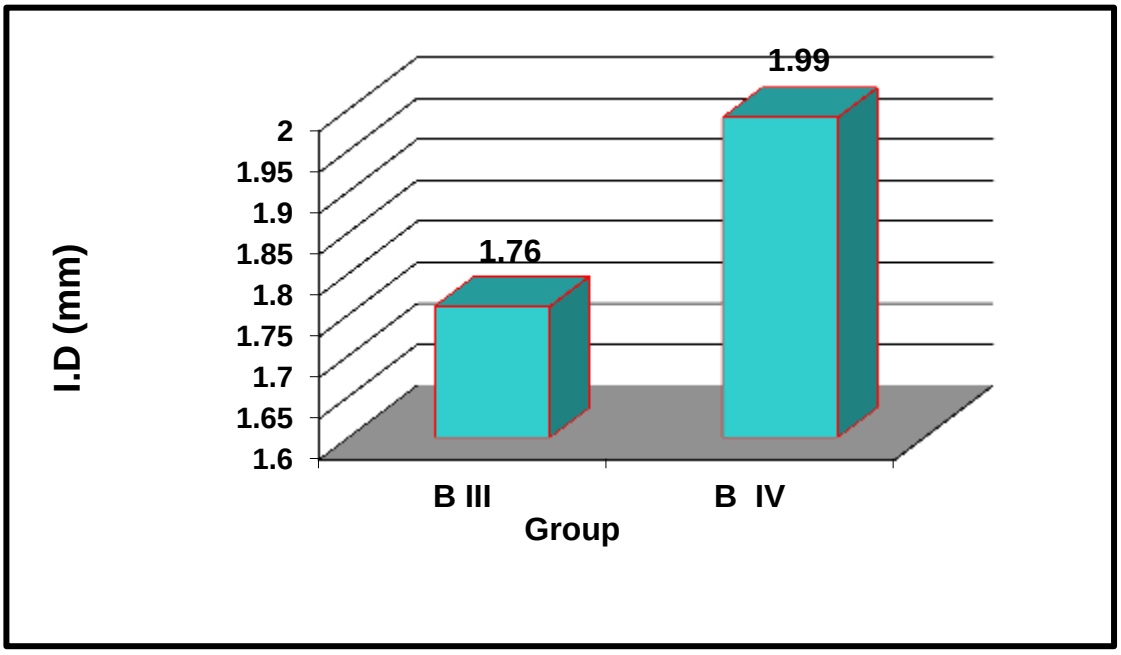

Figure 3-17 1 Bar chart of means incision depth, BIII = incision assisted with normal saline, BIV= incision assisted with methylene blue 1%.

2. Incision width

There no significant difference in the incision width between BIII and BIV, as shown in table (3-15), figure (3-18).

Table 3-15: Comparison between BIII and BIV in incision width (mm).

Group B	Mean ± SD of incision width (mm)	t-test	p-value
BIII	1.09 ± 0.37	0.1007	0.1148 NS
BIV	1.17 ± 0.44		

(NS) Non significant, (*) significant, (**) highly significant

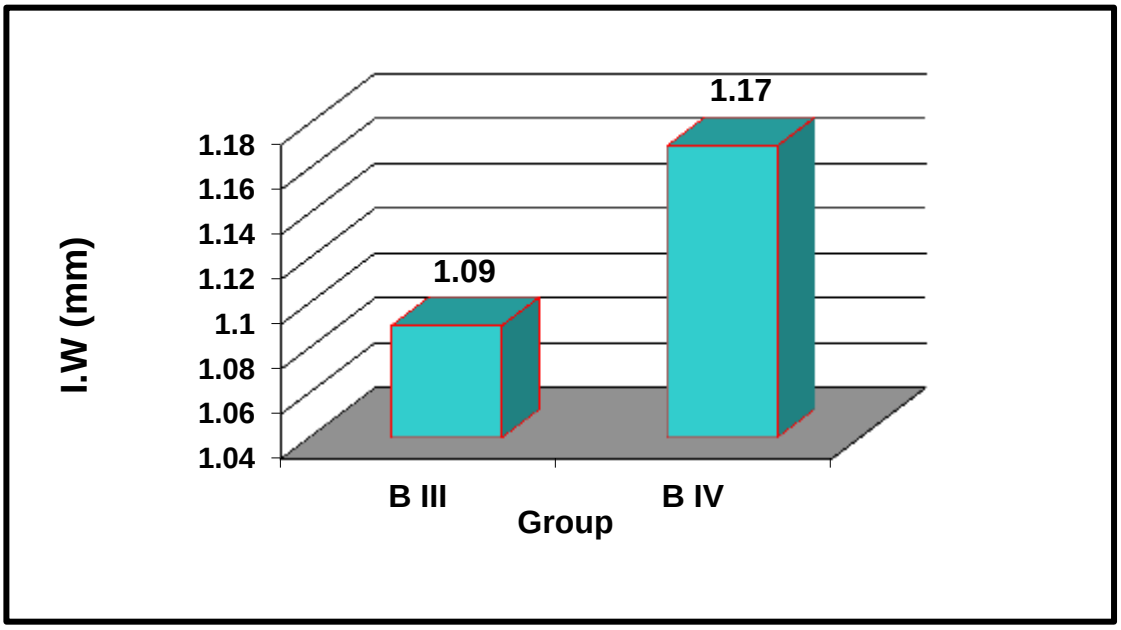

Figure 3-18 1 Bar chart of means incision width, BIII = incision assisted with normal saline, BIV= incision assisted with methylene blue 1%.

3. Damage depth

There is a high significant difference between BIII and BIV in the damage depth with P<0.01 as shown in table (3-16), figure (3-19).

Table 3-16: Comparison between BIII and BIV in damage depth (mm).

Group B	Mean ± SD of damage depth (mm)	t-test	p-value
BIII	1.42 ± 0.26	0.0087	0.0001**
BIV	1.14 ± 0.22		

(NS) Non significant, (*) significant, (**) highly significant

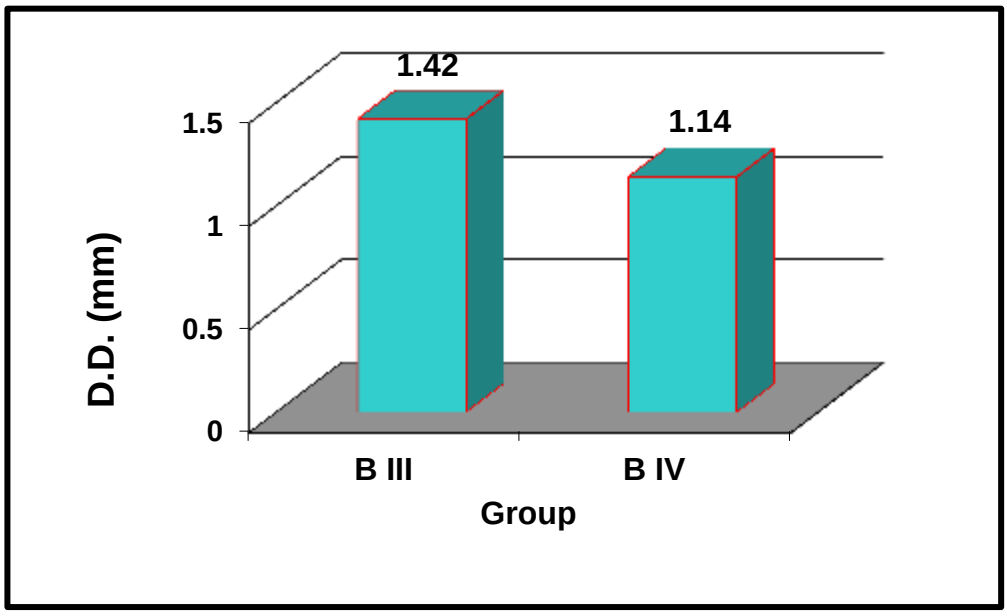

Figure 3-19 1 Bar chart of means damage depth, BIII = incision assisted with normal saline, BIV= incision assisted with methylene blue 1%.

4. Damage width

There is a high significant difference between B III and B IV in damage width with P<0.01 as shown in table (3-17), figure (3-20).

Table 3-17: Comparison between B III and B IV in damage width (mm).

Group B	Mean ± SD of damage width (mm)	t-test	p-value
BIII	1.30 ± 0.23	0.0749	0.0001**
BIV	1.06 ± 0.19		

(NS) Non significant, (*) significant, (**) highly significant

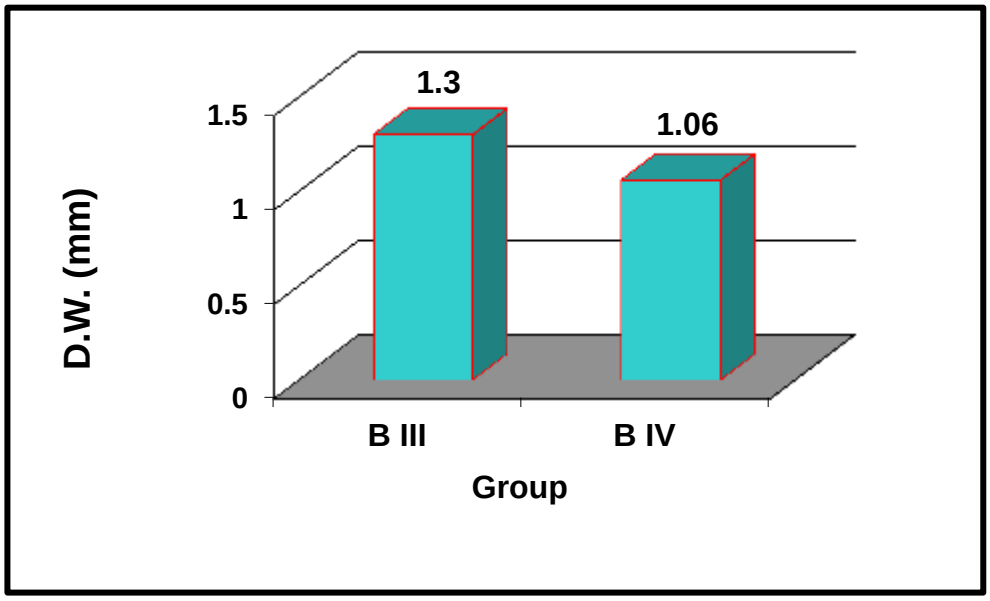

Figure 3-20 1 Bar chart of means damage width, BIII = incision assisted with normal saline, BIV= incision assisted with methylene blue 1%.

5. Regularity and quality scale

The result shows that that no significant difference in (0) scale and there is a high significant difference in (<2, ≥2, 4) scale with p-value<0.01 between BIII and BIV table (3-18)

Table 3-18 Distribution of sample according to Regularity and quality between BIII and BIV

Regularity and quality	B III: (%)	B IV: (%)	P-value
0	0 (0.00%)	0 (0.00%)	NS
<2	4 (40.00%)	2 (10.00%)	0.0001 **
≥2	5 (50.00%)	6 (30.00%)	0.0074 **
4	1 (10.00%)	12 (60.00%)	0.0001 **
Total No.	10	20	--
** (P≤0.01)-Highly Sig.			

3.2. Discussion

The lasers have been approved as an alternate to conventional surgical procedures over last 10 years, due to more efficient performance and less morbidity in comparison to scalpel. (Azma and Safavi, 2013, Verma et al., 2012).

Diode lasers wavelengths between 800-980 nm with high absorption in chromophores pigmented tissues hemoglobin, melanin and collagen, make them perfect soft tissue laser that can be safely used around the dental hard tissue because their weak absorption by water and hydroxyapatite (Sobouti et al., 2014).

The risk of thermal damage is drawback of using laser that resulted from heating tissue by laser energy. Each type of laser creates a degree of thermal damage by photothermal effect on target tissue. When the laser hit the biological tissue the laser energy that transmitted through the tissue increases the temperature of tissue at the incidence point above the 100 °C. (Jasiński, 2010).

This an in vitro study aimed to evaluate (time, properties and thermal damage) of incisions made before application of erythrosine stain 3% as external chromophoers with diode laser 940 nm as used by Agrwal et al (2018), and methylene blue stain 1% with diode laser 810 nm for first time as external chromophoers in this study.

The methylene blue stain has been used in different medical application such as treatment of malaria, as photosensitizer for PDT to treat selected cases of plaque psoriasis and in periodontics as mouth wash to treat periodontitis (Aka et al., 2015).

The incision time

The result showing a highly significant difference between all stained and unstained samples for both groups used erythrosine stain 3% and methylene blue stain 1%.

Many studies stated that the exposure time of laser is an important factor during tissue radiation by laser. As the time increase the heat accumulation increase and may end with undesirable thermal effect. Time is one of the critical parameters along with laser power and specific absorption of tissue that affecting thermal damage. (Kishen and Asundi, 2007).

Using the external chromophores in this study result in decrease the incision time as the infra-red laser beam is highly absorbed by targeting oral soft tissue chromophores as hemoglobin and melanin which are exist internally within the tissue and by dark pigments (erythrosine, methylene blue) that are applied externally on the tissue, so the laser tissue interaction is begins directly when laser reach the target chromophores that exist on the surface of tissue. (Sotoode et al., 2015).

The histological evaluation

The histological evaluation procedure used in this study is commonly applied for examination of incision properties and thermal effect on oral soft tissue. The in vitro model utilize the tongue tissue which considered the criterion for examine laser cutting efficiency, as this model used by (Wilder et al., 1995, Vaderhobli et al., 2010).

The result of this study showing that there is a significant difference in depth and width of incisions for samples stained with methylene blue stain, while for groups stained with erythrosine stain was not significant difference. For the thermal lateral damage there was a highly significant difference in the thermal damage both vertically and horizontally for both groups stained with erythrosine and methylene blue stains.

This decrease in the thermal damage resulted when target chromophoers are matching or near the applied laser wavelength, the laser beam will be absorbed with high percentage rather than scattered and target chromophores acted as photoreceptors that applied externally on the tissue (Sankaran et al., 2002).

Recent studies concluded that diode laser induced the baggiest and deepest epithelial and sub-epithelial damage and thermal tissues artifacts on both layer of oral mucosa. They preferred the use of scalpel which cause less tissue damage and no thermal that produced by diode laser (Monteiro et al., 2019, Protasio et al., 2019).

And even with hemostatic ability of diode laser more tissue damage occurred and extra inflammatory cell infiltration in site of incisions than with scalpel. The scalpel blade provide healing and no effect on histological analysis of specimen but unable to produce hemostasis that specially needed in highly vascular lesion (Abdul Wahab et al., 2020).

Also the healing after removal of same oral lesion founded to be faster clinically and histologically with scalpel blade than with diode laser due to undesirable thermal heating of tissue by laser (Cayan et al., 2019).

It advised for the excision of oral lesion by diode laser should be with 5 mm diameter for pathologist to make an accurate histological diagnosis, because the diode laser can cause irreversible thermal effect in lesion with diameter smaller than 3 mm (Angiero et al., 2012).

All these studies showing the thermal damage drawback of laser on tissue, but with result of this in vitro study overcomes this problem as incision time and thermal damage decreased by using the external chromophores.

The results of regularity and quality of incision margins. Showing that highest quality incision were for all groups stained with both erythrosine and methylene blue stains, that appear as sharp, regular and well defined incision

margins, which is agreed with the result of (Agrwal et al. 2018) that stated that the most regular incisions were for erythrosine 3% stained group. And also agreed with result of (Azevedo et al., 2016) that stated more regular incisions are these appeared with minimal thermal damage.

When the selected wavelength absorbed with high degree from water based chromophores, result in sharp, well defined, smooth incision with minimal sub surface disturbances (parker 2007).

As the relationship between exposure time, absorption, and thermal damage, state the selection of proper wavelength that matching existing chromophores weather it present endogenously inside tissue like melanin and hemoglobin or artificial pigments added exogenously (Elanchezhiyan et al., 2013).

3.3. Conclusion

Using the erythrosine 3% with diode 940 nm laser and methylene blue 1% stain with diode 810 nm laser as external chromophores before cutting oral soft tissue in vitro resulted in:

1. Decreasing the incision time significantly.
2. Minimizing the thermal damage both vertically and horizontally.
3. Produce more regular incision margins.

3.4. Suggestions for future wok

1. Increase the number of in vitro samples
2. Manipulation of used parameters like another wavelength, exposure time, power of laser, different concentration of stains.
3. Comparison between pulsed and C.W modes with application of stains for tissue cutting.
4. Utilize the skin of rabbit as vivo animal model and examine the tissue healing after incisions made with application of stains.
5. Use another biologically accepted stain
6. Use different time for application of stains

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الخلاصة

المقدمة: أصبح الدايود ليزر مستخدمًا على نطاق واسع في العمليات الجراحية للأنسجة الفموية الرخوة ، نظرًا للعلاقة القريبة بين أطوال الموجات تحت الحمراء وأمتصاص صبغات الأنسجة الفموية الرخوة. يعد الضرر الحراري أحد الآثار غير المرغوب فيها التي تنتج عن تسخين الأنسجة بالليزر.

الهدف: التقييم النسيجي المرضي مختبريا لتأثير دايود ليزر (٩٤٠ و ٨١١٠) نانومتير على خصائص الشقوق و التأثير الحراري عند القطع بأستخدام حاملات الالوان الخارجية (الأرثروسين ٣% والمثيلين الأزرق١%).

المواد والطرق: ١٢٠ عينة بأبعاد (١,٥ × ١ × ٠,٥) سم من ستة ألسنة اغنام جمعت مباشرة بعد ذبح الحيوانات. قسمت الى مجموعتين (أ و ب). مجموعة أو ٦٠ عينة تمت الشقوق بأستخدام دايود ليزر ٩٤٠ نانومتر، ٢ واط، موجة مستمرة طرف جراحي مفعل. هذه المجموعة قسمت الى أ١ (٢٠ عينة) تمت الشقوق فيها مع ماء ملحي، في حين أ٢ (٤٠ عينة) الشقوق تمت بمساعدة الأرثروسين ٣% . مجمجوعة ب، ٦٠ عينة تمت الشقوق بأستخدام دايود ليزر ٨١٠ نانومتر،(١,٥ ، ٢,٥) واط، موجة مستمرة، طرف جراحي مفعل. هذه المجموعة قسمت الى ب١ (١٠ عينات، ١,٥ واط) و ب٣ (١٠ عينات، ٢,٥ واط) تمت الشقوق فيها بأستخدام الماء الملحي، ب٢ (٢٠ عينة، ١,٥ واط) و ب٤ (٢٠ عينة ٢,٥ واط) تمت الشقوق فيها بأستخدام الميثلين الأزرق ١%.

النتائج :

أظهرت النتائج فرق معنوي عالي في زمن الشق مع فرق احصائي < ٠,٠١ ، فرق معنوي عالي في عمق وعرض الضرر الحراري مع فرق احصائي < ٠,٠١ وفروق معنوية عالية في حواف الشقوق زمن الشق مع فرق احصائي < ٠,٠١ لجميع العينات التي صبغت ب (الارثروسين ٣% و الميثيلين الازرق١%) في كلا المجموعتين أ وب.

الاستنتاج : مقارنة مع قطع الليزر للأنسجة الغير مصبغة، استخدام حاملات الأصباغ الخارجية يمكن أن ينتج عنه تقليل وقت القطع ، وتقليل الضرر الحراري عموديا وأفقياً وإنتاج حواف شقوق أكثر انتظامًا.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
معهد الليزر للدراسات العليا

كفاءة قطع دايود ليزر (٩٤٠و٨١٠) نانومتر للانسجة الفموية الرخوة بمساعدة حاملات الالوان الخارجية: دراسة نسيجية مرضية

رسالة مقدمة إلى

معهد الليزر للدراسات العليا / جامعة بغداد / لأستكمال متطلبات نيل

شهادة ماجستير علوم في الليزر / طب الأسنان

من قبل

علي محمد صادق

بكالوريوس طب وجراحة الفم والاسنان ٢٠١٢

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بكالوريوس طب وجراحة الفم والاسنان

بورد جراحة الوجه والفكين

دبلوم عالي ليزر في طب الاسنان

١٤٤٢هـ **٢٠٢١ م**