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Effects of 940nm diode laser with or without varnish on dentinal tubules and dentin permeability (In vitro 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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﴿ وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ﴾

صدق الله العظيم

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Dedication

To my husband for his endless support

To my mother and father with love

And to the whole of my family

Zahraa

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Abstract

Introduction: One of the most anomalous features of the pulp-dentin complex is its hypersensitivity. It was found that hypersensitive teeth have a larger number and wider patent tubules than those of non-sensitive teeth. Laser has been suggested for hypersensitivity treatment, the effect of lasers on dentinal tubules differ depending on the wavelengths, irradiation parameters, and application techniques.

Objectives: The aims of this study were to compare between the effects of 940 nm diode laser at different power densities, with and without 5% sodium fluoride white varnish with tri-calcium phosphate, on the sealing the end of exposed dentinal tubules, and on the dentin permeability, and to suggest the safe parameter settings of 940 nm diode laser in dentinal tubules sealing to dental tissues.

Materials and methods: Two hundred-sixteen human upper premolar teeth were used. Samples were divided into 10 groups: the control group, varnish only group, laser without and with varnish groups at 0.8, 1.6, 2 and 3 W (power densities: 323.8, 647, 809.7, 1214.57 W/cm^2 respectively), exposure duration: 10 seconds, optical fiber diameter: 300 µm; continuous and noncontact mode). Ninety-six teeth were used to assess the temperature change during laser irradiation, 10 samples per group for pulp chamber temperature measurement, 2 samples per group for external surface measurement. Hundred teeth, ten per group, were used to examine dentin permeability by measuring dye penetration depth along the dentin. Twenty of specimens, two per group, were observed under Scanning Electron Microscope for surface morphology analysis with 2000x, and 5000x magnification.

Results: Measurements of the pulp chamber temperature elevation showed that maximum temperature increase was 3˚C, with an average dentin thickness of 1.871 mm. For external surface temperature elevation the results revealed that temperature rise, for both laser alone and with varnish groups, was between (67-97.9 ◦C) at 1.6, 2 W, and it exceed 200 °C at 3W which indicate surface carbonization was occurred. For dentin permeability measurement, the result revealed that there is a positive significant difference between control group and 1.6Wwith varnish, 2W , 3W groups (P< 0.05), while the highly positive significant difference seen between control group and 2W with varnish, 3W with varnish groups (p< 0.01) both showed the same significant difference, and no significant difference for the varnish alone group. Scanning electron microscope analysis revealed that dentinal tubules diameter reduction was observed in 1.6W, 2W, and 1.6W with varnish groups, while approximately optimum sealing of dentinal tubules was occurred in 2W with varnish group. But for 3W groups carbonization was noticed.

Conclusions: The combined application of 940 nm diode laser at 2W with 809.7 W/cm² power density, with 5% sodium fluoride white varnish with tri-calcium phosphate showed a significant improvement in their effects on the dentinal tubules sealing as proved with SEM micrographs and on the dentin permeability reduction as displayed by diminished dye penetration depth, compared to each treatment alone.

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List of abbreviations

Abbreviations	Term
ANOVA	Analysis of Variance
A	Ampere (unit of electric current)
ºC	Degree Celsius (unit of temperature)
CW	Continuous wave
DEJ	Dentinoenamel Junction
DH	Dentin hypersensitivity
DT	Dentinal tubules
E	Energy
EDTA	Ethylene diamine tetraacetic acid
Er:YAG	Erbium- doped: Yttrium, Aluminum Garnet
Er, Cr: YSGG	Erbium, Chromium: Yttrium-Scandium-Gallium Garnet
FDA	Food and Drug Administration
F	Fahrenheit (unit of temperature)
ft	Feet (unit of length)
GaAlAs	Gallium–aluminum–arsenide
He-Ne	Helium –Neon laser
HS	Hypersensitivity
Hz	Hertz (unit of frequency)
IR	Infra-Red
J	Joule (Energy unit)
J/cm²	Joule per square centimeter (unit of energy density)
KTP	Potassium Titanyl Phosphate
L	Wavelength
LLLT	Low level laser therapy
M	Mole (Unit of amount of substance)
MP	Maximum power
mW	Mill watt = 10^{-3} W
W/mK	Watt per meter kelvin (unit of conductivity)
nm	Nanometer (= 10 -9 m)
Nd:YAG	Neodymium doped Yttrium –Aluminum Garnet
P	Power
PD	Power density
PDT	Photodynamic therapy

rpm	Revolution per minute (measure of rotational speed)
pH	Power of hydrogen
s	Second (unit of time)
S.D	Standard Deviation
S.E	Standard Error
SEM	Scanning electron microscope
UV	Ultra violet
V	Volt (the electrical unit of voltage)
W	Watt (unit of power)
W/cm^2	Watt per square centimeter (unit of power density)

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Chapter One

Introduction and Basic Concepts

Chapter One

1.1 Introduction

1.1.1 Dentin hypersensitivity

One of the most anomalous features of the pulp-dentin complex is its hypersensitivity. The extreme sensitivity of this complex is difficult to clarify (1). Dentin hypersensitivity is described as a short, sharp, well-localized pain in response to thermal, tactile, osmotic, or chemical stimuli which cannot be attributed to any other form of dental defect or pathology (2).It is better to regard as a symptom complex, rather than a disease. It occurs as a result of exposed dentin (3). It was found that hypersensitive teeth have a larger number of patent tubules and wider tubules than those of non-sensitive teeth (1)

1.1.1.1 Theories of Dentin hypersensitivity

Three mechanisms have been suggested to explain dentin hypersensitivity:

1-Direct Innervation Theory

The theory states that, dentin comprises nerve endings which extend to the dentin-enamel junction and respond when it is stimulated(1), but there is no evidence that outer dentin, which is the most sensitive part, is innervated (4)For this reason, the theory is no longer accepted.

2- Odontoblast Receptor Theory

The second theory to explain dentin hypersensitivity suggest the odontoblast to be a receptor cell. This concept has been considered due to the fact that the odontoblast is of neural crest origin, so it has an ability to transduce and conduct an impulse (1). The lack of this concept, as has argued by different studies, that odontoblasts are matrix forming cells and therefore they are not considered to be nervous cells, and no synapses have been demonstrated between odontoblasts and pulpal nerves (5).

3-Hydrodynamic Theory

The third mechanism suggested to explain dentin hypersensitivity involves movement of fluid inside the dentinal tubules. This fluid movement through the tubule activates the free nerve endings in the plexus of Raschkow (1)The excitation of pulpal nerves, depends upon the intensity of stimuli (5)These stimuli could be cooling, drying and application of hypertonic chemical materials. About 75% of patients with DH complain of pain with application of cold stimuli (6)

In spite of the concept that fluid movement through the DTs produces pain, it should take in consideration that not all exposed dentin is sensitive. The "hypersensitive" dentin has more widely patent tubules as compared with "non-sensitive" dentin. The wider tubules increase the fluid movement and consequently the pain response (7, 8)(see figure 1-1)

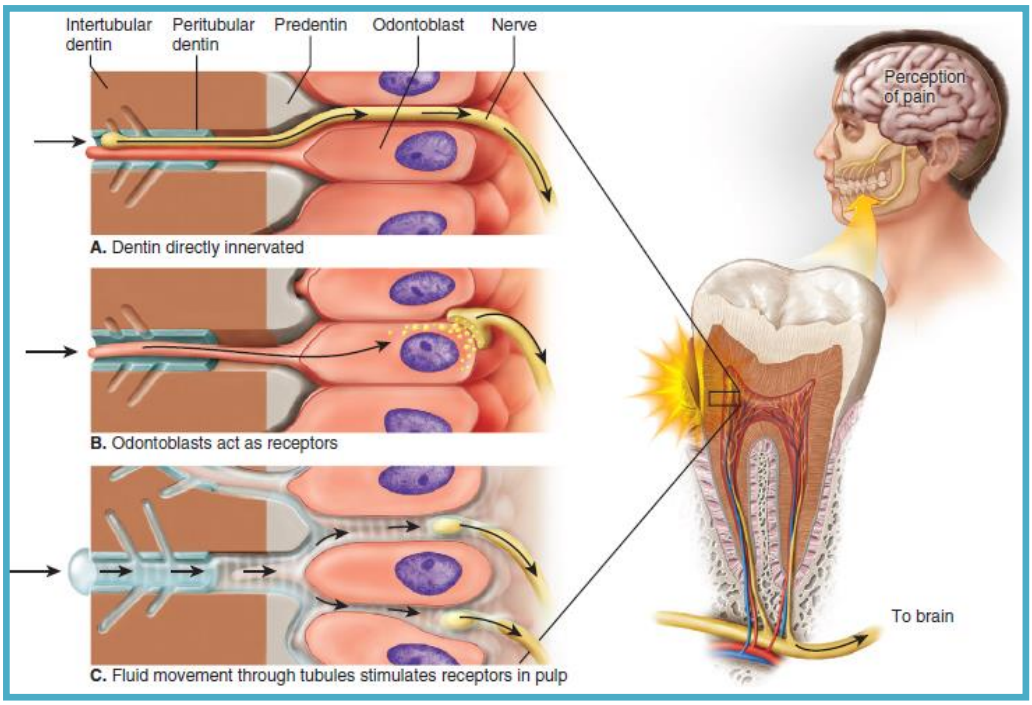
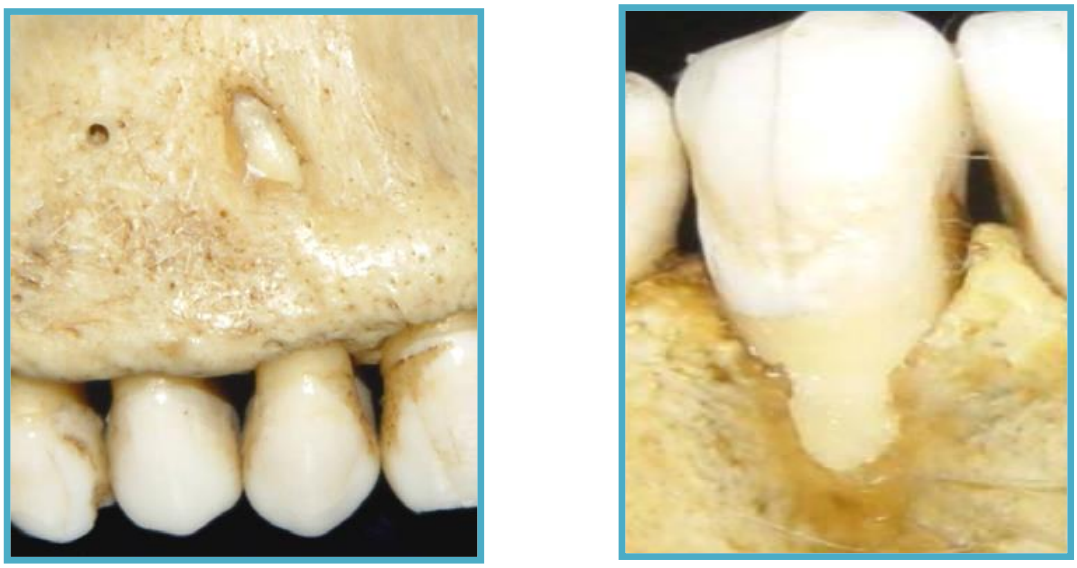


Figure 1-1: Three theories of dentin hypersensitivity. *A* Direct Innervation Theory. *B* Odontoblast Receptor Theory *C* Hydrodynamic Theory (1).

1.1.1.2 Etiology and predisposing factors of dentin hypersensitivity

There are many causes for dentin hypersensitivity. Enamel loss and cementum denudation, which lead to dentin exposure, are the major contributing factors. Causes also comprise gingival recession, which could be due to root prominence and thin overlying mucosa, frenum pulls, periodontal disease, acute or chronic trauma, dehiscence or fenestration (figure 1-2), and orthodontic movement (9) (10).As enamel is lost and/or recession is existing, the exposed cementum and/or dentin are abraded and eroded easier than enamel due to their lower inorganic mineral content compared to enamel, dentin abrades by 25 times and cementum abrades by 35 times, faster than enamel(11).



(a) (b)

Figure 1-2: Fenestration (a) and dehiscence (b) (12)

Loss of enamel or cementum may occur as a result of one or usually a combination of two or more of these factors (2):

1- Attrition: is a mechanical wear of the incisal or occlusal surface of the teeth usually associated with occlusal functions (13)while excessive pathologic wear is resulted from parafunctional habits, such as bruxism (2)

2- Abrasion: is an abnormal tooth surface loss as a result of direct friction forces between the teeth and external objects, most commonly resulted from improper tooth brushing which is usually seen as a sharp, V-shaped notch in the gingival portion of the facial aspect of the tooth (13)

However, tooth brushing by itself, has slight effect on enamel. Actually the combination of tooth brushing and erosive agents lead to loss of tooth structure (14).

3- Erosion: is a chemical tooth surface dissolution (13)due to exposure to nonbacterial acids in the diet, chemical products, drugs or endogenous acids from reflux or regurgitation of stomach acid (2)

4- Abfraction: is a mechanical wear of the cervical surface of the teeth (15)It can be seen as a cervical, wedge-shaped defects due to flexure of cervical area under a heavy eccentric occlusal force resulting in micro fractures (13) (Figure 1-3).

5- Bleaching: the main adverse effect of vital tooth bleaching is post-dental bleaching hypersensitivity, which is mainly related to the penetration of the bleaching agent into the pulp chamber (15)Frequently, higher concentrations of peroxide cause a greater degree of hypersensitivity. (16).

6- Periodontal Treatment: supra and/or subgingival scaling can lead to dentin exposure which cause teeth hypersensitivity (15)

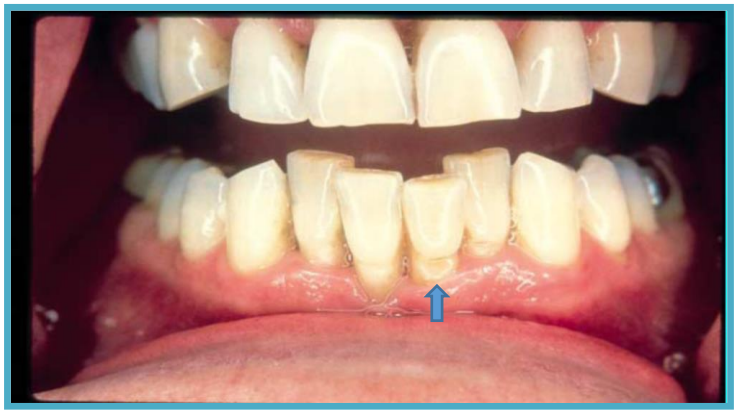


Figure 1-3: Cervical abfraction on the mandibular left incisor (arrow) (17).

1.1.1.3 Prevalence of Dentin Hypersensitivity

Dentin hypersensitivity is a predominant oral problem, affecting about 40% of adults worldwide (18). Some studies have stated prevalence levels as high as 68% (19). Higher incidences occur in 20 to 40 year-old patients (20).Studies have revealed that premolars are most commonly affected teeth(21).However, another study has reported that mandibular incisors are most commonly affected(22). DH most commonly noticed at the labial or buccal surfaces of teeth, that accounting for 90% of sensitive surfaces (4). The cervical area of teeth is the most affected site (13).

1.1.1.4 Diagnosis of dentin hypersensitivity

Diagnosis of DH is confused to most dental professionals. This is due to the fact that several etiologic factors cause hypersensitivity, such as caries, leaking restorations or fractures. Teeth with vital pulps may display symptoms that are identical to DH. The conclusive diagnosis is more challenging when clinical causes of reversible pulpitis are existing in combination with exposed dentin(15)

In order to conclude a definitive diagnosis of DH, it is essential to take a clinical history, clinical examination and radiographs. Also it must be taken in consideration, whether the pain (sharp, dull, or throbbing), number of involved teeth and their location, excitable area of the tooth and the pain intensity (2)

Although hypersensitive teeth and reversible pulpitis may present the same symptoms (such as hypersensitivity to cold, heat and air) (3), pulpal pain tends to be dull, and poorly localized and usually persists longer than the applied stimulus (2)

1.1.2 Dentin-pulp complex

1.1.2.1 Dentin

Dentin is a hard tissue that forms the bulk of the tooth. Dentin is a bonelike matrix characterized by several closely packed dentinal tubules that cross its entire thickness and comprise the cytoplasmic extensions of

odontoblasts that are responsible dentin formation and then its maintenance. The cell bodies of the odontoblasts are arranged along the inner side of the dentin, where they also form the periphery of the dental pulp.

1.1.2.1.1Chemical Composition

Mature dentin is made up of approximately 70% inorganic substance, 20% organic substance and 10% of water. The inorganic component of dentin constitutes mainly of hydroxyapatite in the form of plates with some amorphous calcium-phosphate. The organic component is about 90% collagen (mainly type I with small amounts of types III and V) with slight inclusions of different non-collagenous matrix proteins (mucopolysacharades) and lipids (1).

1.1.2.1.2 Histology of dentin

When the dentin is examined microscopically, several structural features can be recognized: peritubular dentin, intertubular dentin and dentinal tubules.

- **Peritubular dentin**

Tubules are bordered by a collar of highly calcified matrix called peritubular dentin (see Figure 1-4). The mechanism of formation and its specific composition are still unknown, in comparison to intertubular dentin, peritubular dentin has been revealed to be hyper mineralized. Also, peritubular dentin contains few collagen fibrils and seems to be enriched in noncollagenous proteins.

- **Intertubular dentin**

Intertubular dentin is referred to dentin located between the dentinal tubules. It is the primary formative product of the odontoblasts and comprises an interwoven network of type I collagen fibrils in which apatite crystals are deposited. The ground substance involves noncollagenous proteins that is suitable to calcified tissues and some plasma proteins. (1)

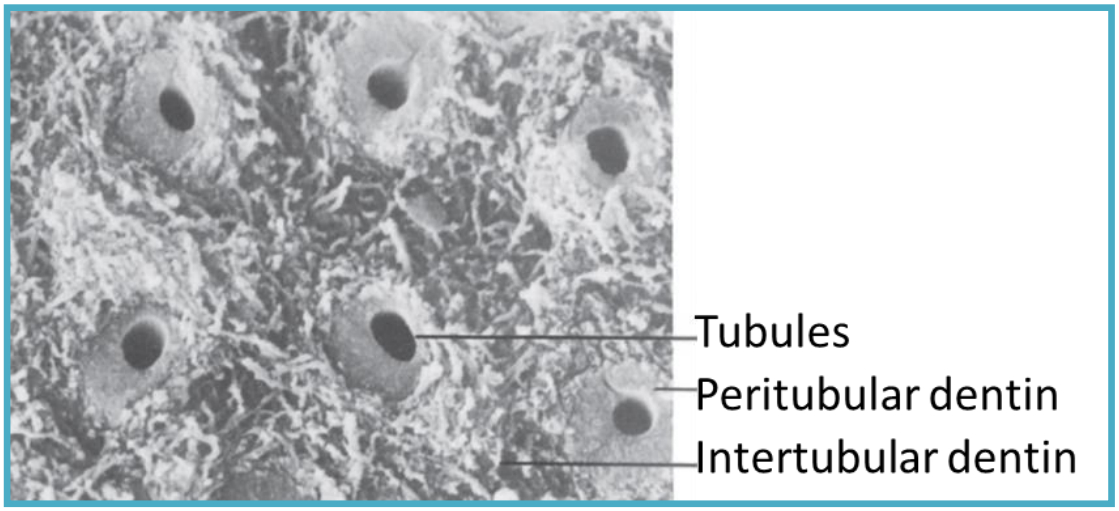
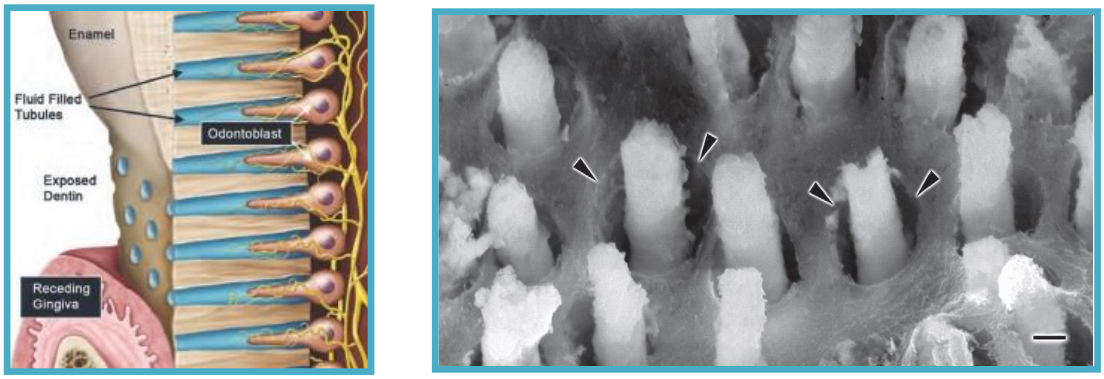


Figure 1-4: Scanning electron microscope view for dentin. (1)

- **Dentinal tubules**

Dentinal tubules are canaliculi that traverse the dentin layer where odontoblast processes run through. DTs extend through the entire thickness of the dentin from the dentin-enamel junction or the dentin-cementum junction to the pulp, form a network for the distribution of nutrients throughout dentin. The tubules follow an S-shaped curvature from the outer surface of the dentin to the perimeter of the pulp. This configuration of the tubules indicates the path taken by the odontoblasts during dentinogenesis. The dentinal tubules are tapered in shape being largest diameter near the pulp and thinnest at the dentinoenamel junction (1)(see figure1-5). It was found that the diameter of tubules is about 2.5 μm at the pulp and about 0.9 μm peripherally, with numbers of approximately 45,000/mm² at the pulp, 29,500/mm² in the middle dentin, and 20,000/mm² peripherally as investigated by scanning electron microscopic (23).



(a) **(b)**

Figure 1-5: Dentinal tubules transverse view (a), Dentinal tubules comprise the processes of the odontoblasts (arrowheads) (b) (1)

The DTs house the processes of the odontoblasts that comprises few organelles like endoplasmic reticulum, Golgi apparatus and mitochondria (24) (see figure1-6). Perioodontoblastic and postodontoblastic spaces are assumed to be filled with extracellular dentinal fluid, which involve potassium and sodium ions(25)

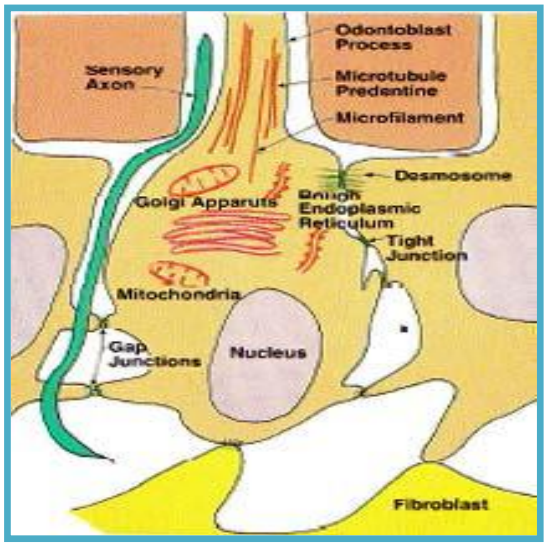


Figure 1-6: The ultrastructure of odontoblasts show numerous organelles in the body of the cell (25)

Dentinal tubules could be blocked physiologically with calcified material of sclerotic dentin. When this occurs in multiple tubules in the same area, the dentin appears glassy and becomes translucent (Figure1-7). The

deposition of sclerotic dentin increases with age and is mostly noticed in the apical third of the root and between the dentinoenamel junction and the surface of the pulp in the crown. The occlusion of dentinal tubules with mineral may initiates at approximately 18-year-old premolars without any detectable external influence; therefore it was assumed that sclerotic dentin is a physiologic response and that occlusion is achieved by continued deposition of peritubular dentin (1)

Figure 1-7: Scanning electron microscope view for sclerotic dentin (arrow)(26)

1.1.2.2 Pulp

The dental pulp is the soft connective tissue that supports the dentin. (1)

1.1.2.2.1 Chemical composition

It made up of 75% water and 25% organic material. It contains a mixture of cells embedded in an extracellular matrix of collagen fibers (mainly type I and type III collagen) and ground substance which is composed of glycosaminoglycan, glycoprotein, and water(25).

1.1.2.2.2 Pulp histology

When its histologic appearance is inspected, four distinctive zones can be distinguished: (1) the odontoblastic zone at the pulp periphery (2) a cell-free zone underneath the odontoblasts (3) a cell-rich zone, where

cell density is high, which is seen adjacent to the cell-free zone and (4) the pulp core, which is characterized by the chief vessels and nerves of the pulp (see figure 1-8)

The prime cells of the pulp are the odontoblasts, fibroblasts, macrophages, undifferentiated ectomesenchymal cells, and other immune competent cells.(1)

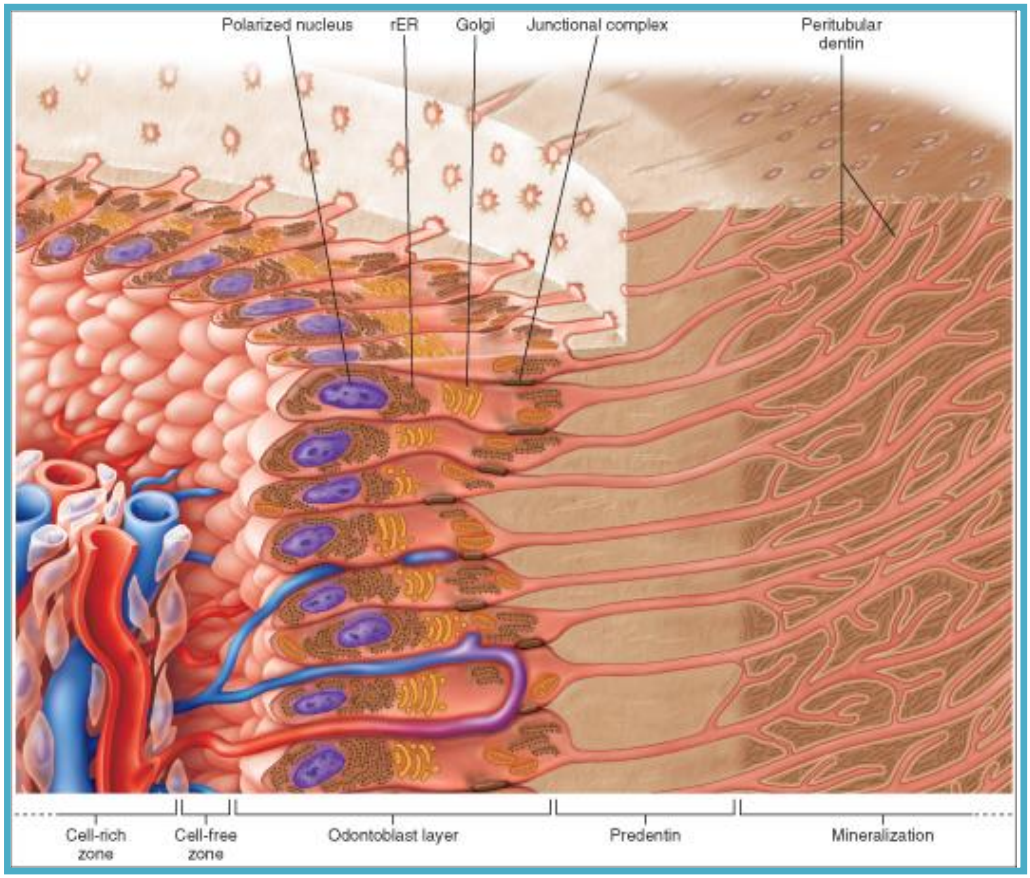


Figure 1-8: Schematic representation of pulp zones. (1)

1.1.2.2.3 Innervation of the dentin-pulp complex

The dental pulp is richly innervated. Nerves enter the pulp through the apical foramen, alongside with afferent blood vessels, as they pass through the pulp core, they branch out to form the plexus of Raschkow at cell-free zone just beneath the cell bodies of the odontoblasts (Figure1-9) (1)

Pulp innervation primarily consist of sensory afferent nerves of the trigeminal (fifth cranial) nerve and sympathetic branches from the superior cervical ganglion. Each bundle comprises myelinated axons (δ delta and beta

β), which relate to sharp pain, and unmyelinated axons (C fiber), that relate to dull pain (see figure 1-10)(25, 27).

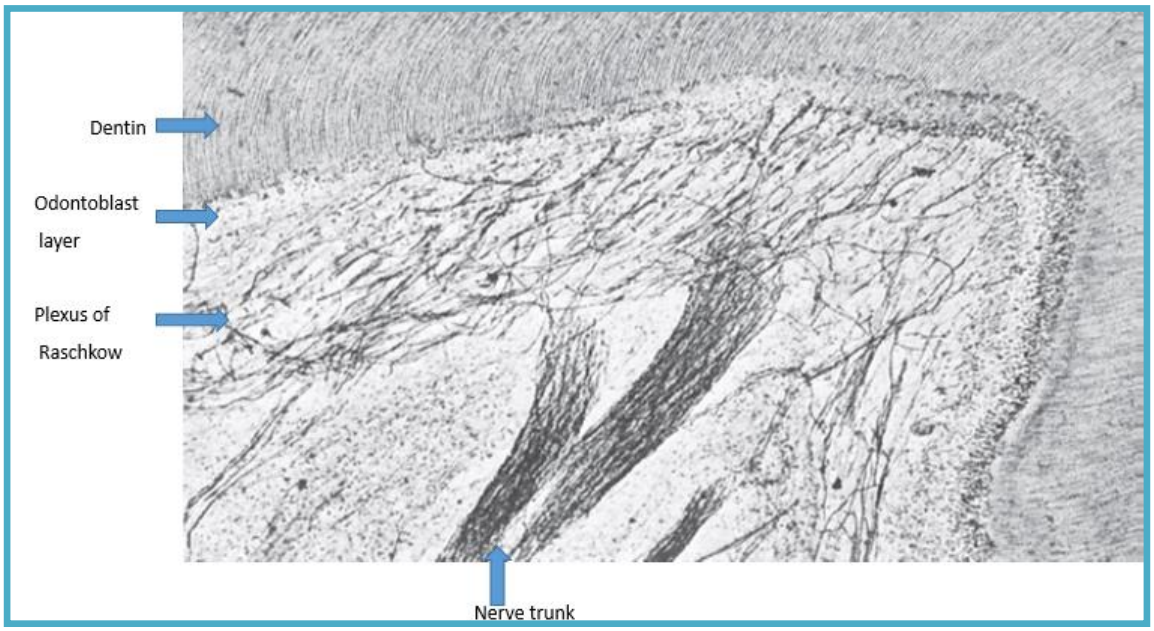


Figure 1-9: Plexus of Raschkow in a silver-stained section. The ascending nerve trunks branch to form this plexus, that is situated beneath the odontoblast layer (1)

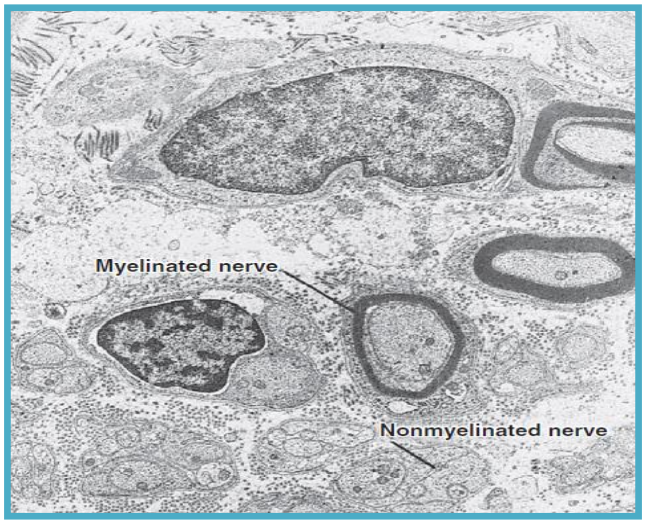


Figure 1-10: Electron micrograph showing a mixture of myelinated and nonmyelinated nerves in pulp(1).

1.1.3 Cementum

It is a hard layer of a calcified tissue that forms the outer covering of the tooth root. Cementum extends from the cement-enamel junction to the apex of the tooth, which seals the open DTs of root dentin and provides an attachment for periodontal ligament with bone.(28).

1.1.3.1 Chemical composition

It composed of 45% inorganic material, 35% organic material and 20% water

1.1.3.2 Histology of cementum

Cementum varies in thickness at different levels of the root. It is thickest at the root apex 150-200 μm and in the interradicular areas of multirooted teeth and thinnest cervically 16-60 μm(28) (figure 1-11).

Two types of cells are found within cementum: cementoblasts, and cementocytes. Cementum is classified based on the presence or absence of cementocytes cells within its matrix into:

1- Cellular cementum which extends from the middle to apical third of the root and furcations.
2- Acellular cementum which extends from cervical margin to the apical third of the root(1).

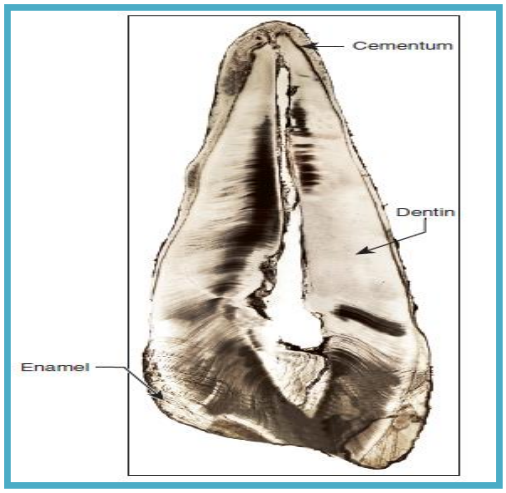


Figure 1-11: Ground section of a premolar showing showing variable thickness of cementum around the root (1).

1.1.4 Dentin permeability

Permeability states the ease of passage or the diffusion rate of fluids, bacteria, ions and minute particles through tissue under standard conditions. Many factors influence this passage, including, thickness, structure and chemistry of the involved tissue, the exposed area and the pressure wielded on the process(24).

In concern to dentin permeability, it mainly depends on dentin exposure and dentinal tubules patency(25). Tubules may be occluded by growth of peritubular dentin or by mineral deposits. Such deposits reduce the size of the lumen and they may occur physiologically as an age change, or as a consequence of external influences such as caries, restorative procedures, or exposure to the oral environment(24).

The smear layer, which is comprised of a deposit of salivary proteins, debris and remnants of calcified materials, may plug the opening of the tubules leading to a reduction of the dentin permeability, but they are easily and rapidly removed by acid etching , chelating agents (8)(see figure 1-12)

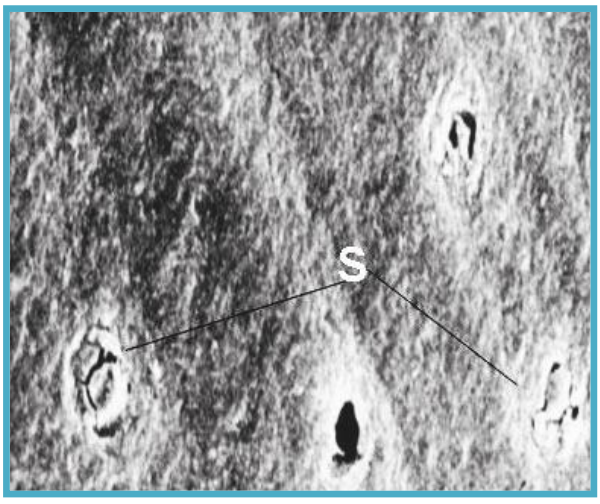


Figure 1-12: Scanning electron micrographs showing cross-section of a crown surface with a cavity preparation. Note a smear plug (S) of the dentinal tubules(24).

1.1.5 Conventional treatment of dentin hypersensitivity

Dentin hypersensitivity is a common clinical complaint that is difficult to treat because the treatment outcome is not reliably successful(13). Current methods for treatment may be only temporary effective and consequences are not always predictable(2).An ideal desensitizing agent should be effective, not irritant to the pulp, relatively painless, being easy to apply and spread, rapid action, and permanently effective, and should not cause teeth discoloration(29)

1.1.5.1 Classification of Desensitizing Agents

Desensitizing agents have been classified according to their mechanism of action or by their mode of administration.

The treatment methods of dentin hypersensitivity can be categorized into two groups by their mode of action (2):

- Tubular occlusion
- Blockage of nerve activity

For dentinal tubules occlusion, ingredients may act by (30):

1-Protein precipitation, like:

- Glutaraldehyde
- Silver nitrate
- Zinc chloride
- Strontium chloride hexahydrate

2-Plugging dentinal tubules, like:

- Sodium fluoride
- Stannous fluoride
- Strontium chloride
- Potassium oxalate
- Calcium phosphate
- Calcium carbonate
- Bioactive glasses

3-Sealing dentinal tubules, like:

- Fluoride varnishes
- Oxalic acid and resin
- Glass ionomer cements
- Composites
- Dentin bonding agents

While agents that interfere with the transmission of the nerve impulse act by raising the extracellular potassium ion concentrations and affecting nerve polarization. When potassium is sustained for a period of time, the nerve excitation is reduced and the nerve becomes less sensitive to the stimuli(31).

Potassium salts (potassium nitrate, potassium chloride or potassium citrate) are commonly used for this method. Various clinical studies have revealed the efficacy of potassium salts in decreasing DH(32, 33).

But it is difficult to categorize desensitizing agents by their mode of action, because in the case of products with more than one substance, their desensitizing action has not yet been well clarified, hence it may be easier to classify them by their mode of administration(15):

- At home desensitizing therapy
- In office desensitizing therapy

For at home (self-applied) desensitizing therapy, desensitizing agents include toothpastes, mouth washes and chewing gums(34). It has been revealed that toothpastes containing 5% potassium nitrate and 0.454% stannous fluoride significantly decrease the DH. As well as, toothpastes comprising potassium nitrate and fluorides have been shown to reduce post-bleaching hypersensitivity(35, 36). Mouthwashes and chewing gums containing potassium citrate, potassium nitrate or sodium fluoride are also recommended (34). The outcome of at-home desensitizing therapy should

be reevaluated after every 3-4 weeks. If it is not successful in relieving DH, in-office therapy should be began (30).

In office (professional-applied) desensitizing therapy, several desensitizing agents could be used, such as glass ionomer cement, bonding agent, composites, fluoride varnishes, and oxalates. It was found that they were capable of reducing dentin hypersensitivity(37).

1.1.5.2 Sodium Fluoride White Varnish 5% with TCP

NaF white varnish is a fluoride-containing varnish with calcium and phosphate**.** It could be applied to enamel and dentin. The product is saliva-activated. It contains a modified rosin that is virtually invisible when applied to the teeth. It is indicated to treat hypersensitive teeth. After NaF white varnish application to the tooth surface, the rosin slowly dissolves and releases fluoride, calcium and phosphorus ions into saliva. Fluoride and calcium react to form calcium fluoride which aids in hypersensitivity reduction. The calcium in NaF white varnish increases the likelihood of forming calcium fluoride globules on tooth surfaces (38).

1.1.6 Laser Basics

Laser is an acronym for Light Amplification by Stimulated Emission of Radiation. Lasers are devices that generate and amplify light by the process of stimulated emission (39).

1.1.6.1 Elements of the Laser System

1-Active Medium

The active medium is composed of atoms, molecules, or compounds. Lasers are commonly named for the material of the active medium, which could be **(1)** gas, such as in a CO2 laser; **(2)** a solid crystal, like in Er: YAG or Nd: YAG laser; **(3)** a solid state semiconductor, like in diode lasers; or **(4)**a liquid, like in pulsed dye laser.

2-Pumping source

It refers to excitation source, which could be a flash lamp, electrical circuit, or chemical pumping systems, when this pumping source

pumps energy into atoms or molecules of the active medium, the energy is absorbed by the electrons enabling them to be raised to an excited state and creating a population inversion.

3- Optical Resonator

It consists of two mirrors located at each end of the optical cavity, parallel to each other; while in case of a semiconductor, it is composed of two polished surfaces at each end. These mirrors or polished surfaces act as optical resonators, reflecting the waves back and forth, enabling collimation and amplification the developing beam. A cooling system, focusing lenses, and other controlling mechanisms may combined the mechanical components(40).(Figure 1-13)

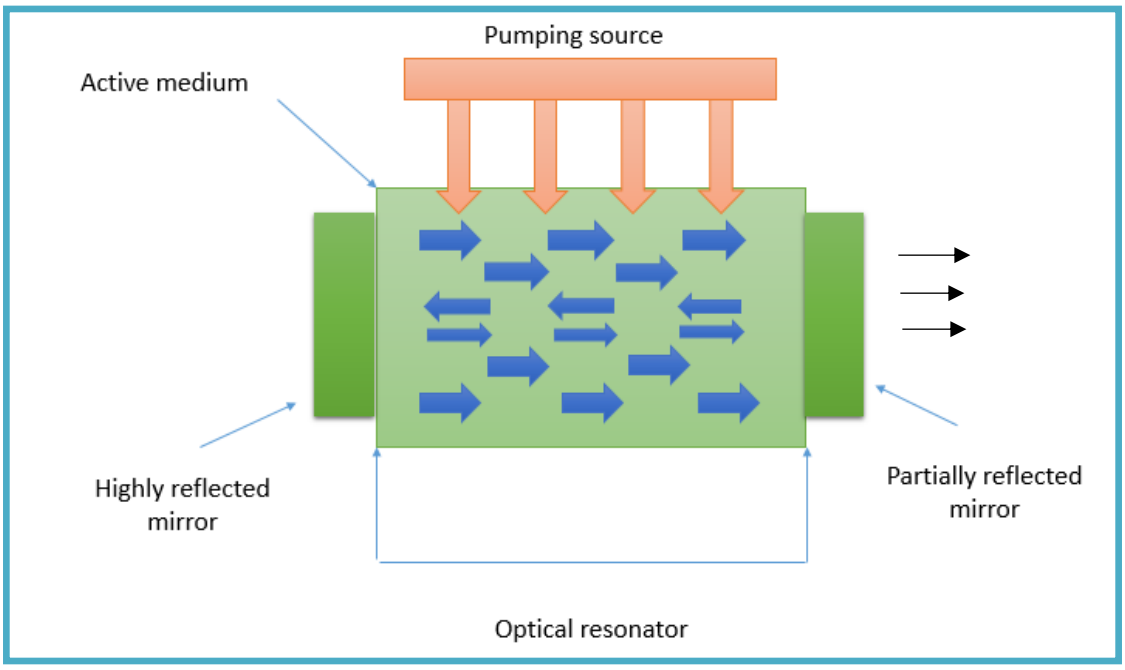


Figure 1-13: Schematic illustration of main components of laser system.

1.1.6.2 Properties of Laser Light

1-Coherence

Coherence literally means in phase. A coherent light field is, therefore, a field of light, in which the oscillations are overlap in space and time(41). This result in the production of a specific form of focused electromagnetic energy(40).

2-Collimation

Collimated light refers to light in which all of its rays are traveling parallel to each other in a definite direction (unidirectional). Lasers generate the most collimated light of any type of light source, so it can travel over a long distance with the least divergence(40).

3-Monochromaticity

Laser light is monochromatic because it produces a beam of a single wavelength, which is either visible or invisible depending on its wavelength if it is inside or outside the visible portion of the spectrum(40). Monochromaticity means how pure in color (frequency or wavelength) the laser beam is(42).

4-Intensity or Brightness

Intensity is the power of the laser beam divided by the cross-sectional area of the beam. It is accordingly given in watts per square centimeter (W/cm2). It is a measure of the amount of energy that can be applied to a definite region within a given amount of time(42).

1.1.6.3 Laser Operation Modes

Dental laser devices can emit light energy in two modes as a function of time: continuous or pulsed mode. The pulsed lasers can be further divided into gated and free-running modes in delivering energy to the target tissue. Thus, three different emission modes are described, as follows(40):

1. Continuous-wave mode, means that the beam is emitted at only one power level as long as the operator depresses the foot switch.

2. Gated-pulse mode, means that there are periodic chopping of the laser energy, like a blinking light. This mode is attained by opening and closing of a mechanical shutter in front of the beam path of a continuous-wave emission. Most surgical devices that operate in continuous mode have this gated- pulse feature as short as microseconds or milliseconds.

3. Free-running pulsed mode, occasionally referred to as true-pulsed mode. This emission is unique in that large peak energies of laser light are emitted for usually microseconds, followed by a relatively long time in which the laser is off. Consequently, high peak powers, in hundreds or thousands of watts, with low average powers are produced. Free-running pulsed devices cannot have a continuous-wave or gated pulse.

1.1.6.4 Laser Tissue Interactions

Depending on tissue optical properties, laser light energy may have four different interactions with the target tissue (Figure 1-14.), as follows (43):

• **Reflection** means simply the beam being redirected off the surface, with no effect on the target tissue. This reflection can be dangerous because the energy could be redirected to an unintended target, such as the eyes, this is a chief safety concern for laser operation.

• **Scattering** is the shifting of photons directions, resulting in increased absorption due to increased chances of interacting with the principal chromophore. Scattering of the laser beam may cause heat transfer to the adjacent tissue.

• **Absorption** is the attenuation of the electromagnetic wave when it passes through the tissue, which is the usual desirable effect. The amount of energy absorbed by the tissue depends on the on the laser wavelength and the tissue characteristics, such as pigmentation and water content.

• **Transmission** is the transfer of the laser energy directly through the tissue, with no effect on the target tissue. Only non-reflected, non-absorbed or scattered photons will be transmitted through the tissue.

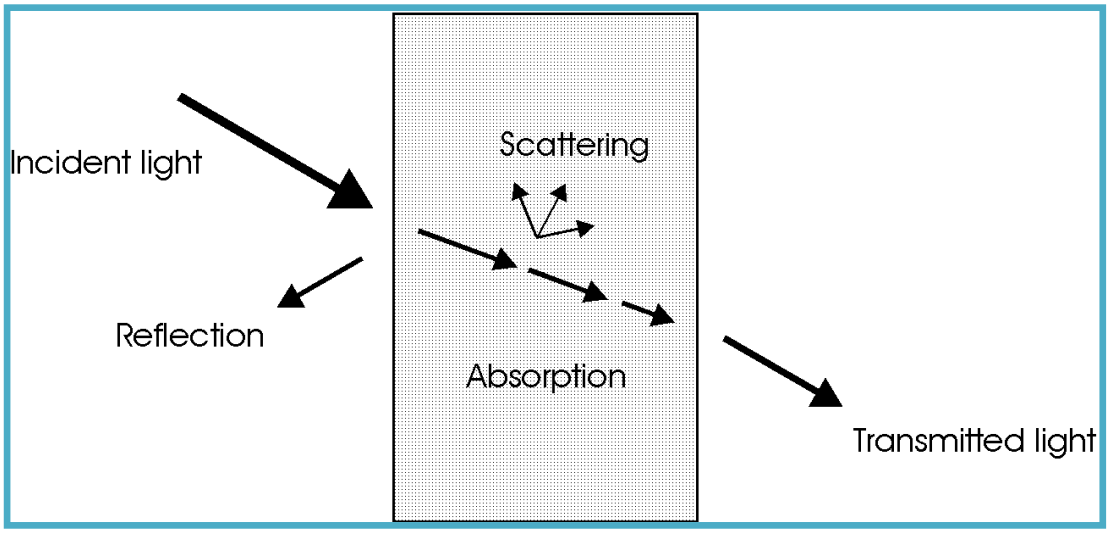


Figure 1-14: Geometry of the four different laser tissue interactions (44).

1.1.6.5 Laser tissue interaction mechanisms

Laser tissue interaction mechanisms can be classified according to wavelength dependence, as illustrated in (figure1-15), into:

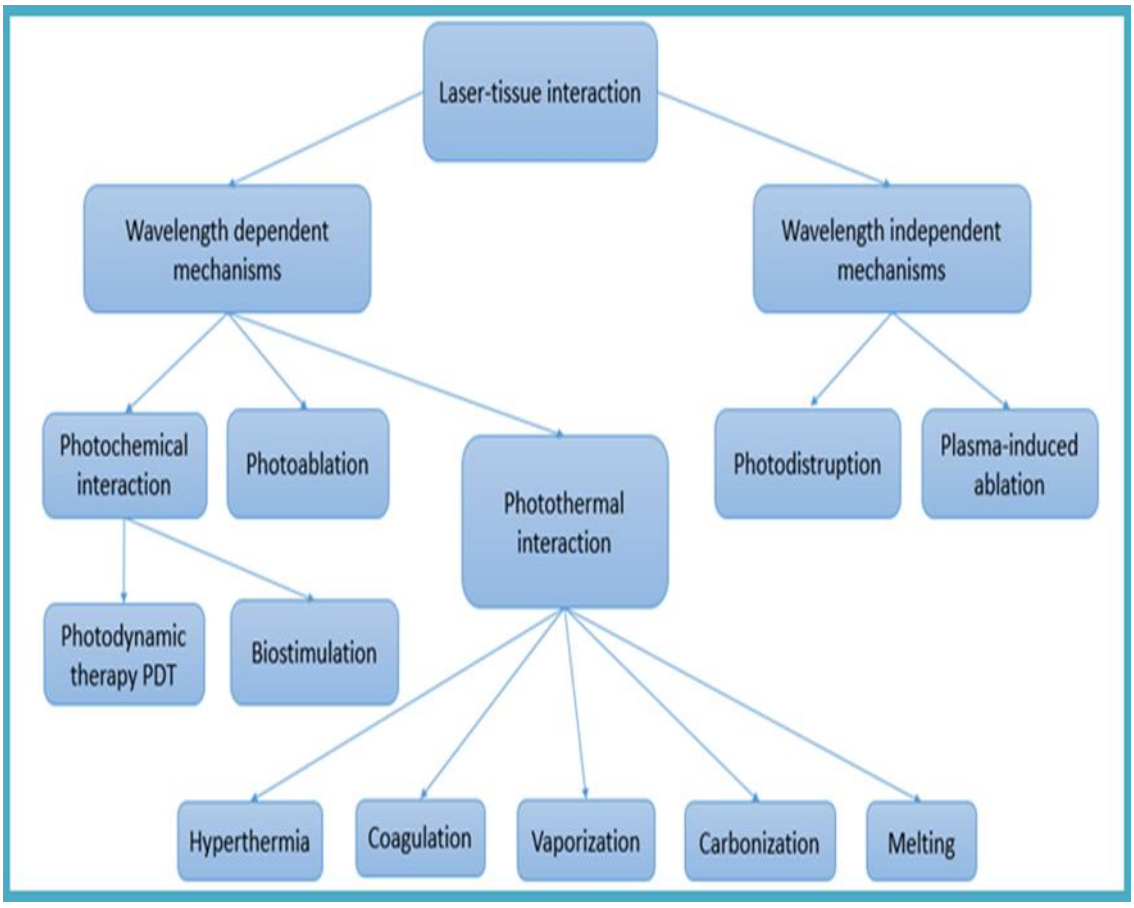


Figure 1-15: Mechanisms of laser interactions with biological tissues

1.1.6.5.1 Wavelength Dependent Mechanisms:

1. photochemical Interaction

The group of photochemical interactions arises from perception that light can induce chemical effects and reactions within tissues or macromolecules. Photochemical interaction mechanisms play a vital part during biostimulation and photodynamic therapy (PDT).

Photochemical interactions occur at very low power densities (typically 1 W/cm2) and long irradiation times ranging from seconds to continuous emission. Careful choice of laser parameters harvests a radiation dispersion inside the tissue that is determined by scattering (43).

a- Photodynamic Therapy

The photodynamic therapy reaction is mediated by exogenous spectrally adapted chromophores called photosensitizers. These photosensitizers, which are capable of causing light induced reactions, are injected into the body. Laser irradiation may then elicit selective photochemical reactions. At the end extremely cytotoxic reactants are released causing an irreversible oxidation of essential cell structures, hence this kind of interaction is also called photosensitized oxidation. Photodynamic therapy is a major modality for cancer treatment(44).

b- Biostimulation Therapy

This process is supposed to occur at extremely low laser power. It is related to enhancing certain metabolic pathways in the living cells, for example, wound healing, anti-inflammation and pain relief. In the injuries zones, acidic condition are usually created preventing proliferation. The exposure to red or near infrared laser light at low power might act as a stimulus to increase cell proliferation and optimum performance (44).

2. Photothermal Interaction

Thermal interaction term stands for a large group of interaction kinds, where the increase in local temperature is the significant parameter

change. In biological tissue, absorption is chiefly due to the presence of free water molecules, proteins, pigments, and other macromolecules.

However, based on the duration and peak value of the tissue temperature achieved, different effects like coagulation, vaporization, carbonization, and melting may be distinguished. At temperatures above approximately 60˚ C, proteins and collagen begin to denature without any vaporization of the underlying tissue. At 100˚ C water vaporization of tissue achieved. These phenomena are very useful in medical applications without affecting the healthy tissue, as long as the tissue temperature can be controlled.(45)

Conversely, if tissue temperature is raised to >200, it is dehydrated and then burned, carbon is the end product. Tissue carbonization occurs when improper laser parameters are used(40). At temperature rise beyond 300˚ C, tissue melting occur.

3. Photoablation Therapy

Srinivasan and Mayne-Banton are the first to discover photoablation (1982). They identified it as ablative photodecomposition. A type of UV light-induced ablation characterized by the removal of tissue in a very clean and exact manner without any appearance of thermal damage such as coagulation or vaporization. This means that the material is decomposed when exposed to high laser intensity. Typical threshold values of this kind of interaction are 10^7 –10^8 W/cm^2 at laser pulse durations 10-100 ns. By the absorption UV photon, the energy gain exceed the chemical bond energy of the tissue molecules, leading to bond breakage causing photoablation due to the volume stress.By the absorption of visible and infra-red photon, the energy gain will not exceed the chemical bond energy of the tissue molecules, so ablation will not occur unless photons add up in energy leading to global rise in temperature and noticeable thermal effect either vaporization or melting, this type of ablation is called thermal decomposition(44).

For dental hard tissue ablation, the primary interaction occurs at 100˚C when the water is converted to steam whose increased volume causes an explosive expansion and removal of that tissue (46, 47).

1.1.6.5.2 Wavelength Independent Mechanism:

When using power densities beyond 10^{11} W/cm² in fluids and solids or 10^{14} W/cm² in air, with a pulse duration in femtosecond to nanosecond range, multiphoton ionization of atoms and molecules may occur, and a phenomenon called Optical breakdown takes place. The physical effects related with optical breakdown are plasma formation and shockwave generation. If breakdown happen inside soft tissues or fluids, Cavitation and jet formation may furthermore arise (44).

Since the interaction time in femtosecond to nanosecond range, which is faster than most of the thermal excitation modes, the nature of this process is primarily electronic.(48)Besides, the wavelength is not a significant parameter(49).

The important characteristic of optical breakdown is that the possibility to deposit an energy not only in pigmented tissue but also in low absorbing medium. This is due to a high absorption coefficient of the induced plasma (44).

In the photodisruption, the tissue is fragmented by mechanical forces. In plasma-induced ablation, the effect is spatially restricted to the breakdown site, while in shock wave and cavitation effects spread into adjacent area. Actually, purely plasma induced ablation is not detected in nanosecond pulses, because the threshold energy density of optical breakdown is higher compared to picosecond pulses.

As both interaction mechanisms, plasma-induced ablation and photodisruption, depend on plasma generation, it is not always easy to distinguish between them.

In the 1990s, ultrashort lasers (picosecond and femtosecond pulse duration) were suggested to be used for dental hard tissues ablation with exact tissue removal and reduced thermal or mechanical damages(44).

1.1.6.6 Laser Safety Standards and Hazard Classification

Laser safety classification is based on a possibility to cause biological damage. According to American National Standard Institute (ANSI) Z136.1-2007 Section 3 and Appendix B standard, lasers can be classify into (50)**:**

1-Class 1 Laser System: which is regarded to be incapable of producing damaging radiation levels during operation, therefore it exempt from most control measures.

2-Class 1M Laser System: that is regarded to be incapable of producing hazardous exposure conditions during normal operation unless the beam is viewed with an optical instrument such as an eye-loupe or a telescope.

3- Class 2 Laser System: the emission of this class lasers is in the visible portion of the spectrum (400 to 700 nm), Eye protection is normally afforded by the aversion response.

4- Class 2M Laser System: the emission of this class lasers is in the visible region. It could be possibly hazardous if viewed with certain optical aids.

5- Class 3R Laser System: a laser systems that is possibly hazard if viewed by eyes; directly or by specular reflection, especially if the eyes are focused and stable.

6-Class 3B Laser System: These laser systems may be in visible or invisible regions, with medium powers. They cause potential eye hazard if viewed directly or by reflection. Its scattered radiation is not hazard for skin, except for high power lasers with certain wavelengths

7- Class 4 Laser System: This system involves high powered laser in visible or invisible portion of spectrum. They potentially cause acute hazard for eye and skin in direct, reflected, or scattered exposure. Also they should

have careful hazard attention for fire and byproduct emissions from target materials.

1.1.7 Clinical applications of Lasers in dentistry

Different lasers has been used in dentistry (See table 1-1)

Table 1-1: Lasers used in dentistry with its main applications(51)

Lasers	Wavelength (nm)	Spectrum	Mode	Uses in Dentistry
Nd:YAG (KTP)	532	Visible (green)	Pulsed	-Coagulation/ hemostasis. -Dental bleaching -Composite curing - Caries diagnosis
He-Ne	633	Visible (red)	CW	- Laser Doppler flowmetry - Desensitization - Hemostasis
Diode laser	635 655	Visible (red)	- CW - Gated pulsed	- Caries detection - Biostimulation
Diode laser	810 940 980	Invisible (near infrared spectrum)	- CW - Gated pulsed	- Bacterial decontamination - Soft tissue surgery (incision and ablation) - Desensitization - Periodontal pocket treatment
Nd:YAG	1,064	Invisible (near infrared spectrum)	- Free running pulsed - CW	- Bacterial decontamination - Soft tissue surgery (incision and ablation) - Desensitization - Periodontal pocket treatment
Er:YAG **Er,Cr: YSGG**	2,940 2,780	Invisible (mid infrared spectrum)	- Free running pulsed	- Bacterial decontamination - Soft tissue ablation - Subgingival soft tissue curettage - Scaling and root debridement - Hard tissue conditioning - Hard tissue ablation
CO2	10,600 9,600	Invisible (far infrared spectrum)	- CW - Gated-pulsed	- Soft tissue incision and ablation - Subgingival soft tissue curettage

1.2 Literature review of lasers in dentin hypersensitivity treatment

Laser has been suggested for the treatment of dentin hypersensitivity. The results from previous studies regarding the effect of lasers on the treatment of dentin hypersensitivity differ depending on the wavelengths, irradiation parameters, and application techniques (52).

In some studies, the dentin is irradiated with low power (low level) lasers (He-Ne or diode lasers), it was assumed to act directly on the pulp nerve endings causing analgesia by inducing change in neural transmission within the pulp but they do not alter dentin surface morphology so their effects seem to be transient (53), while for high power (high level) lasers (diode, Nd:YAG, Er:YAG, Er Cr: YSGG, and CO_2 lasers), it was reported that they provoke melting effect with recrystallization of dentine inorganic component resulting in occlusion of dentinal tubules. But actually this does not happen. Later, it was found that laser treatment reduces hypersensitivity by denaturation of protein of dentin organic components that lead to DTs sealing (2). Only the sealing effect is regarded to be durable (54).

- **Helium –Neon laser (He-Ne)**

From the previous investigations, He-Ne laser (wavelength 633nm) with 6mW (low power) for 5 min had been used for the treatment of dentin hypersensitivity (55).But the treatment effectiveness showed only 5.2% to 17.5% as reported by Wilder-Smith et al.(56).

- **Neodymium doped Yttrium Aluminum Garnet laser (Nd: YAG)**

Nd: YAG laser (wavelength 1064nm) has been evaluated for the treatment of DH. The mechanism is supposed that laser-induced sealing or narrowing of DTs, as well as direct nerve analgesia (57).

Farmakis et al. compared between the efficacy of the Nd:YAG at 0.5 W, 10 Hz, and 50 mJ for 30 seconds and a bioglass desensitizing paste (Novamine®).The SEM analysis revealed that the Nd:YAG group displayed higher occlusion of DTs than the Novamine group (58).

Later, Farmakis et al. investigate different Nd:YAG laser powers: 0.5 ,10 Hz, 50 mJ and 1 W, 10 Hz, 100mj ,for 30seconds The result revealed that the 1 W laser, either alone or combined with bioglass desensitizing paste, was more efficient in comparison to 0.5 W laser. (59)

mAl-Khatib et al. reported that Q-switched Nd: YAG laser at 120mJ with 6Hz for 2 minutes, is efficient in sealing exposed dentinal tubules (60).

Al-Saud and Al-Nahedh evaluate different desensitizing pastes (Gluma®, Tenure Quick®, Quell and VivaSens®) and Nd:YAG laser. The result showed that, all of the investigated treatments have a favorable effect in occluding or reducing the dentinal tubules diameter and the best method was Nd: YAG laser group at 0.8W for 1 minute exposure. (61)

A clinical study investigate the difference in decreasing dentin hypersensitivity between Nd:YAG laser (at 1.5 W, 10 Hz, 100 mJ, , four irradiations performed, each for 15 seconds, totaling 60 seconds of irradiation with intervals of 10 seconds) , Gluma® and a combination of them. Although all methods have revealed a marked pain reduction, the combined laser and desensitizing paste was the most significant treatment (62).

Some Authors evaluated the efficacy of a potassium binoxalate gel alone and in combination with Nd: YAG laser at 1 W, 10 Hz, and 60 seconds Scanned Electron Microscope analysis showed that the combined laser treatment is higher in sealing DTs, in spite of the gel appears as an effective aid due to its micro-crystals penetration (63).

White et al. demonstrate that pulsed Nd: YAG irradiation at 2W, 20 Hz for 10 seconds induced pulpal temperature rises of 13.4˚C which lead to harmful pulpal effects. (64).

Abed et al. compared between Nd: YAG laser at 1 W, 10 Hz for 60 seconds, (Seal & Protect™) desensitizing agent and no-treatment.

According to SEM evaluation, it was found that the desensitizing agent was more efficient than laser treatment (65).

Another study, by Lier et al., was reported that the effect of Nd: YAG laser at 4 W for 30 seconds in the treatment of hypersensitive teeth is not different from placebo in pain reduction (66).

- **Erbium doped Yttrium Aluminum Garnet (Er: YAG) and Erbium, Chromium: Yttrium-Scandium-Gallium Garnet (Er, Cr: YSGG) lasers**

A comprehensive analyses reserved to the powerful lasers Er: YAG (2940 nm) and Er, Cr: YSGG (2780 nm). For the treatment of DH, it was found that they are incapable of sealing DTs without causing ablation and carbonization. Instead, they act by altering the nerve fibers, promoting a reduction in the pain caused by DH, that seems to be transient (67).

Aranha and Eduardo used Er, Cr: YSGG laser at different power 0.25-2 W, 20 Hz , each irradiated for 20 s four times, with a 1 minute interval between them. They found that dentinal tubules were only partially closed at 0.25 and 0.5W as well as they noticed carbonization and dentin fracture at higher power (68).

Yilmaz et al. verified immediate pain relief in the Er, Cr :YSGG laser group at 0.25W, 20 Hz for 30 seconds compared to placebo. (69)

Aranha and Eduardo have used Er:YAG laser at 32.4 mJ, 2 Hz for 20 seconds, Er, Cr: YSGG at 0.25 and 0.5 W for 30 seconds. Results revealed that each treatment can reduce the hypersensitivity pain but none can completely eliminate it. Treatment with Er, Cr: YSGG laser at 0.25 W is the most suitable.(67)

An in vitro study combines the Er: YAG laser at 40 mJ, 2Hz for 40 seconds sealing effect with a nano-carbonate apatite tooth paste. The result showed that in the combined group, despite fewer occluded DTs, the layer was thicker than that of the n-CAP alone group. Thicker layer is expected to produce long term effects for DH treatment (70)

- **Carbon dioxide laser (CO_2)**

Using CO_2 laser (wavelength 10600 nm) at moderate energy densities, lead to narrowing or occlusion of DTs, yet there is paucity of studies on its individual use in dentistry in last years.

Romano et al.(71) investigate the sealing ability of the CO2 laser ,at 0.5, 1, 1.5 W for 5 seconds 6 times with 10 s interval, alone or combined with a calcium hydroxide paste. DTs occlusion has been detected in all groups, however, the combination groups demonstrate a higher reduction in hypersensitivity. Besides, dentinal carbonization, cracks had been noticed in samples treated exclusively with CO_2 laser at 1.5 W.

Al-Maliky et al. investigate CO2 laser at 0.65 W for 5seconds 6 times with 20 seconds interval, alone or combined with nanohydroxyaptite paste. They concluded that both groups lead to a reduction of DTs diameter, with a better result in a combination group (72).

However, in a recent study, when dentin surface was evaluated with SEM, morphologic changes like cracks and charring effect of the dentine were observed as a result of CO_2 laser irradiation at 1W for 30seconds (73).

- **Gallium–Aluminum–Arsenide (GaAlAs) diode laser**

Recently, the diode laser (660,795,810,940 and 980 nm) has been the most used laser during daily work. There are many studies about (low power) diode laser, particularly on its success in dentin hypersensitivity treatment (74). It was reported that (low power) diode laser can act directly by depressing neural transmission of stimuli. But long term success in clinical use is questionable (75).

Hashim et al. carried out a clinical study using a diode laser 810nm at 0.5W for 30, 60 seconds. Authors revealed that the sixty seconds exposure time is the most effective. (76)

Frequently, this type of laser had been combined with 3% potassium nitrate, potassium oxalate gel (77-79) at different parameter

setting, like 810 nm at 1.5–2.5mW for 1 min, 660 nm at 30mW for 120 seconds, or with fluoride gels like 810 nm at 500mW, irradiation time of 60 seconds (80) in the hypersensitivity treatment.

In 2012, Romeo et al. used 980 nm diode laser at 0.5 W for 60 seconds, with fluoride gel. The combined treatment showed a marked improvement than fluoride or laser treatment alone.(81)

Aranha et al. compared several products. Gluma® has been applied for 30 seconds, Seal & Protect™ for 20 seconds, 3% Oxa Gel (potassium oxalate) for 2 minutes, APF (acidulated phosphate fluoride) for 1min and last of all, the diode laser treatment 660 nm has been used at 15 mW for 10 seconds exposure time. Even all these modalities have led to a hypersensitivity diminution, laser therapy has longer term desensitizing effects(82)

The recent application of cyanoacrylate has invalidated the diode laser as the excellence therapeutic agent. Flecha et al. have reported that cyanoacrylate has the same efficiency as diode laser 795 nm, at 120mW, applied for 8 seconds (83)

Lin et al. demonstrate that there are no actual differences in terms of pain reduction between (low power) diode laser therapy, different vital desensitization agents or their combination using a network meta-analysis. (84)

A recent in vitro-study focuses on both the sealing ability of dentinal tubules and the potential danger of laser to dental pulp. Umana et al. have used (high power) diode lasers 810,980nm with graphite and concluded that the 0.8 or 1 W laser irradiation for 10seconds can narrow or seal dentinal tubules without damage.(85)

- **Comparison between different lasers**

An in vivo study compared between CO2 laser at 1W for 10 seconds and Er: YAG laser at 60 mJ, 30 Hz for 10 seconds Patients have been treated with only lasers therapy, lasers in combination with fluoride gel

or just fluoride. The best results in pain reduction have been achieved with the combination groups, no superiority was obtained for desensitization among the CO2, Er:YAG alone or combined with fluoride (86).

Another clinical study investigate the effects of CO2 at 2W, 2.7 J/cm^2 and Er: YAG at 40mJ, 10 Hz for lasers in DH discomfort reduction. The result revealed that both lasers are effective in DH treatment, Er:YAG laser group showed more significant effect (87).

Some researchers have found the superiority of Nd: YAG, CO2, and Er: YAG treatment compared to conventional topical desensitizing agents, but between these agents and the diode laser (790,810,830nm), the situation is not well defined (88-90)

In a comparative in vitro study between effects of Er, Cr: YSGG at 0,25W, 20 Hz, Nd: YAG at 1 W, 20 Hz, CO2 at 1 W and 810 nm diode at 2 W lasers, with 1 second irradiation time, on DTs occlusion. A reduction in DTs diameter has been detected in Nd: YAG, Er; Cr: YSGG, and CO2 lasers groups, while the best result was expressed with Nd: YAG laser group (53%). The 810-nm diode laser expressed very slight effect on DTs reduction (91).

A clinical study had been done to assess the effect of Er: YAG at 60 mJ, 2 Hz, for 20 s, Nd: YAG, 100mJ, 15 Hz for 100 s, diode 808 nm at 100mW for20s in DH treatment. The concluded that all of used lasers could be useful to reduce DH, Nd: YAG laser irradiation is the most effective laser(92).

Other authors reported the effectiveness of the diode (660,830nm), Er: YAG and Nd: YAG laser in DH management. But for Er, Cr: YSGG, they noticed placebo effect (93).

Yilmaz et al. in vivo-study, demonstrate the equivalent efficacy of the diode laser 810nm at 8.5 J/cm2 for 60 seconds and the Er, Cr: YSGG laser at 0.25 W, 20 Hz for 30 seconds in DH discomfort reduction. (94)

1.3 Aims of the Study

1- To compare between the effects of 940 nm diode laser at different power 0.8, 1.6, 2, and 3 W, with and without 5% sodium fluoride white varnish with tri-calcium phosphate, on the :

a- Sealing of exposed dentinal tubules by determining the dentin surface morphology using a scanning electron microscope (SEM)

b- Dentin permeability using a stereomicroscope.

2- To suggest the safe parameter settings of 940 nm diode laser in dentinal tubules sealing to dental tissues, using a thermometer and a thermal camera.

Chapter Two
Materials and Methods

Chapter Two

2.1 Materials and equipment

2.1.1 Materials

1. Absolute Ethanol Alcohol (Germany) (figure 2-1 A)
2. Artificial saliva;

- Calcium nitrate (Panreac co. ,Barcelona, Spain)
- Potassium phosphate mono basic (Panreac co., Barcelona ,Spain)
- Potassium chloride (SCRC co., China)
- Cocadylic acid (BDH co., Ltd, Mumbai , India)
- HCl (Central Drug House Co., Ltd, New Delhi, India).

3. Base Plate Wax (Shanghai New Century Dental Materials Co., Ltd. Shanghai, China).
4. Clinpro white varnish (3M ESPE Co., USA)(figure 2-1 B)
5. Diamond disk (22mm diameter, Yuandajgs Co., China)
6. Diamond fissure bur (Mani INC, Japan)
7. Distilled water (Iraq)
8. Ethylenediaminetetraacetic acid 17% (SA Co. Switzerland) (figure 2-1 C)
9. Glutaraldehyde (EOBA CHEMIE PVT, India)
10. Methylene blue (Central Drug House Co., Ltd, New Delhi, India) (figure 2-1 D).
11. Note nail enamel varnish (Italy)
12. Orthodontic wire (0.7 mm, Pigeon dental, France)
13. Periodontal curette (China)
14. Sodium cacodylate(BDH Chemicals Ltd , England)
15. Soft brush (Sensodyne, Glaxo Smirth Kline co., UK)
16. Thermal grease (HT-GY 260, Hutixi co., China). Thermal Conductivity: 1.0 W/mK (figure 2-1 E).
17. Thymol crystals (Oxford co., India)

18. Tubes and racks (India)
19. Two hundred-sixteen sound human maxillary premolar teeth extracted for orthodontic purpose (figure 2-2).

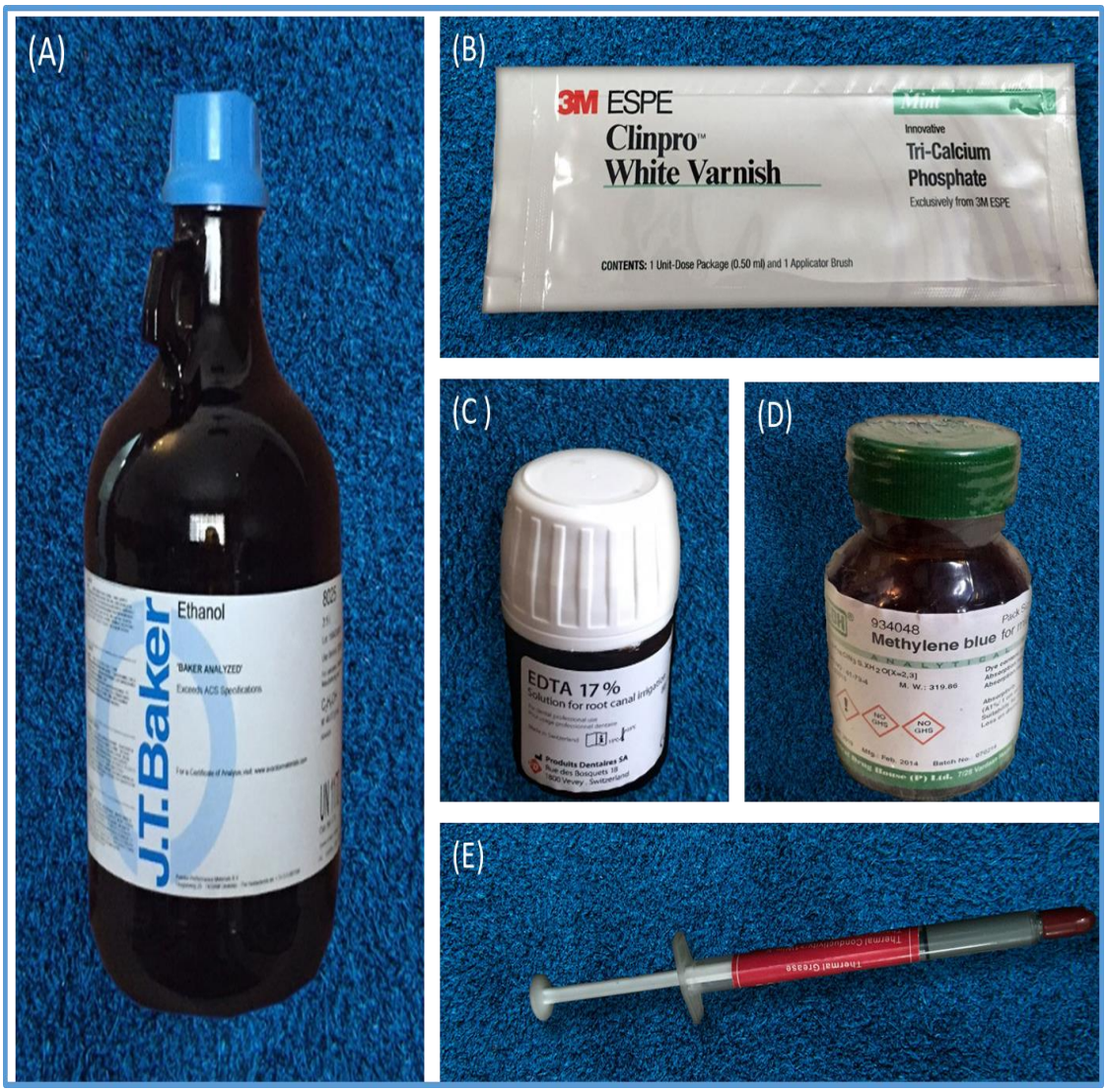


Figure 2-1. Sample of the materials used in the study. (A) Absolute ethanol, (B) Clinpro white varnish, (C) Ethylenediaminetetraacetic acid (EDTA), (D) Methylene blue dye, (E) Thermal grease.

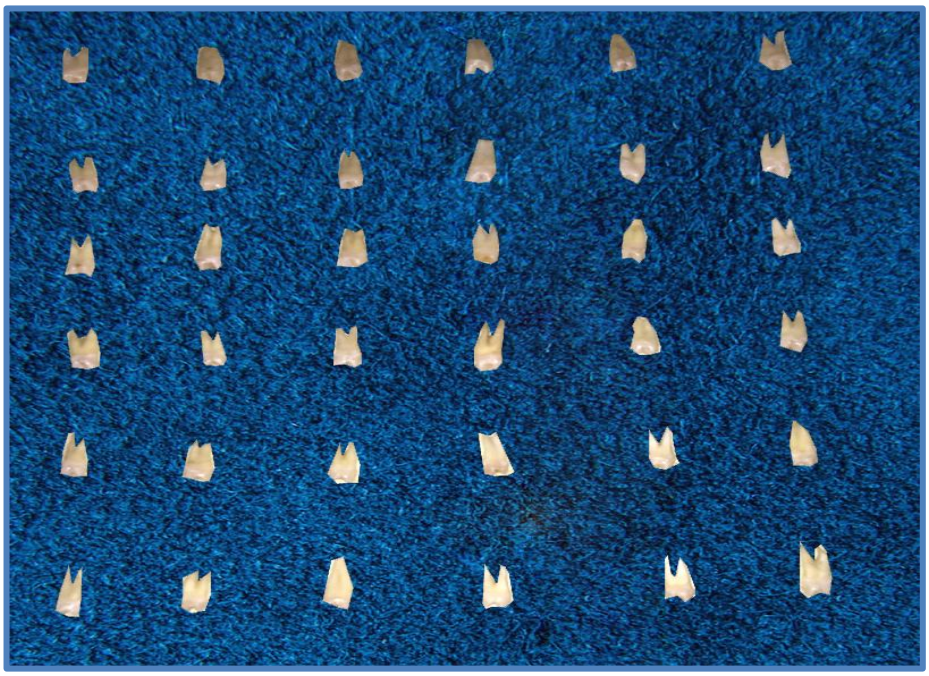


Figure 2-2: Samples of the used teeth in the experiments

2.1.2 Equipment

1. Bench vice (China)
2. Compact plasma sputtering coater (GSL 1100x SPC 12, Quorum Tec., UK) (figure 2-3 A)
3. Lens holder (China)
4. Near-infrared diode laser 940 nm (MP:10 W, CW/pulse), EPIC™ (BIOLASE , San Clement, CA, USA), (figure 2-3 B)
5. PH meter (HANNA instrument, Romania, Europe).
6. Power meter (Coherent Inc., Santa Clara, CA, USA).
7. Protective laser google (Figure 2-3 C)
8. Sensitive Balance (Sartorius co., Gottingen, Germany)
9. Spectrophotometer (UV-9200, Biotech co. Ltd, UK) (figure 2-3 D)
10. Stereomicroscope (Hamilton, Altay Scientific, Italy) (Figure 2-3 E)
11. Scanning Electron Microscope (SEM) (Inspect S50, Czech Republic) (Figure 2-4)
12. Thermal camera (Flir i5). (Figure 2-5 A)

Specifications:

a. Resolution 80 x 80 pixels

b. Accuracy: ±2%

c. Temperature Range -20°C to 250°C

13. Thermometer (AMPROBE TMD®-56, Everett, WA, USA) (figure 2-5 B)

Specifications:

a. Basic accuracy ± 0.05%

b. K type thermocouple.

c. Universal serial bus interface for data logging.

14. Ultrasonic cleaner (CD-7810(A), New Trent, China)

15. Ultrasonic scaler (China).

16. Vernier caliper (TOPEX Sp. Zo. o.S.K., Warsaw, Poland) (figure 2-5 C)

Specification are:

a. Resolution: 0.01 mm.

b. Measurement accuracy: ± 0.02 mm.

17. Water bath (BS-11, JEIO TECH Co, Korea) (figure 2-5 D)

Specifications

a. Bath Volume (L / cu ft): 25 / 0.9

b. Working Temperature Range (C / F): +5 to 100 / +9 to 212

c. Temperature Stability (±C / F): 0.2 / 0.36

d. Electrical Requirements: 230V, 50Hz, 4.6A

Figure 2-3. Some of equipment used in the study. (A) Compact plasma sputtering coater, (B) Near-infrared diode laser 940 nm, (C) protective laser google, (D) Spectrophotometer , (E) Stereomicroscope.

Figure 2-4: Scanning electron microscope

Figure 2-5: Some of equipment used in the study. (A) Thermal camera, (B)Thermometer, (C) Vernier caliper (D) Water bath

2.2 Methods

2.2.1 Sample Collection

Two hundred-sixteen sound extracted human upper premolar teeth with two roots extracted for orthodontic purposes had been used in the present study. The age of patients was between (18-25 years) for standardization. The samples have been washed under distilled water then the soft tissue remnants were removed with air scaler. They were kept in 4°C distilled water containing 0.1% thymol to hinder microbial growth until use (Figure **2-6**).

Figure 2-6: Sample of teeth stored in 4°C distilled water containing 0.1% thymol

2.2.2 Sample Grouping

The samples were grouped into ten groups (12 per group for temperature and thickness measurement (only laser irradiated groups), 2 per group for SEM analysis, ten per group for permeability test) as follows (table 2-1):

1. **Control group:** which was received no treatment to investigate a difference between treated and non-treated DTs in SEM micrographs and permeability test.
2. **Varnish group:** which was regarded as a positive control group. 5% NaF white varnish was applied on the samples surface. This group was not irradiated to examine the difference between irradiated and non-irradiated DTs that were treated with varnish in SEM micrographs and permeability test.
3. **Group (0.8 W):** This group had been irradiated by diode laser 940nm at 0.8 W, the power density is 323.8 W/cm².
4. **Group (1.6 W):** This group had been irradiated by diode laser 940nm at 1.6W, the power density is 647 W/cm².
5. **Group (2 W):** This group had been irradiated by diode laser 940nm at 2 W, the power density is 809.7 W/cm².
6. **Group (3 W):** This group had been irradiated by diode laser 940nm at 3 W, the power density is 1214.57 W/cm².
7. **Group (0.8 W+V):** This group had been irradiated by diode laser 940nm at 0.8 W, the power density is 323.8 W/cm², plus varnish application on the samples.
8. **Group (1.6W +V):** This group had been irradiated by diode laser 940nm at 1.6 W, the power density is 647 W/cm², plus varnish application on the samples.

9. **Group (2W +V):** This group had been irradiated by diode laser 940nm at 2 W, the power density is 809.7 W/cm², plus varnish application on the samples.
10. **Group (3 W+V):** This group had been irradiated by diode laser 940nm at 3 W, the power density is 1214.57 W/cm², plus varnish application on the samples.

All samples for laser alone or with varnish groups, irradiated with diode laser 940nm, which was calibrated before application (Figure 2-8), at a continuous mode, for 10 seconds, in an oblique manner, with a fiber tip diameter: 300µm, 1mm distance between a fiber tip and a samples surface, which was fixed by the aid of orthodontic wire (Figure 2-9)

Table 2-1: Samples grouping and treatment

Groups	Laser	Power	Varnish	Spot size(mm)	Exposure time(sec)	Power density (W/cm^2)
Control	_	_	_	_	_	_
Varnish	_	_	+	_	_	_
0.8W	+	0.8	_	0.561	10	323.8
1.6W	+	1.6	_	0.561	10	647
2W	+	2	_	0.561	10	809.7
3W	+	3	_	0.561	10	1214.57
0.8W+v	+	0.8	+	0.561	10	323.8
1.6W+v	+	1.6	+	0.561	10	647
2W+v	+	2	+	0.561	10	809.7
3W+v	+	3	+	0.561	10	1214.57

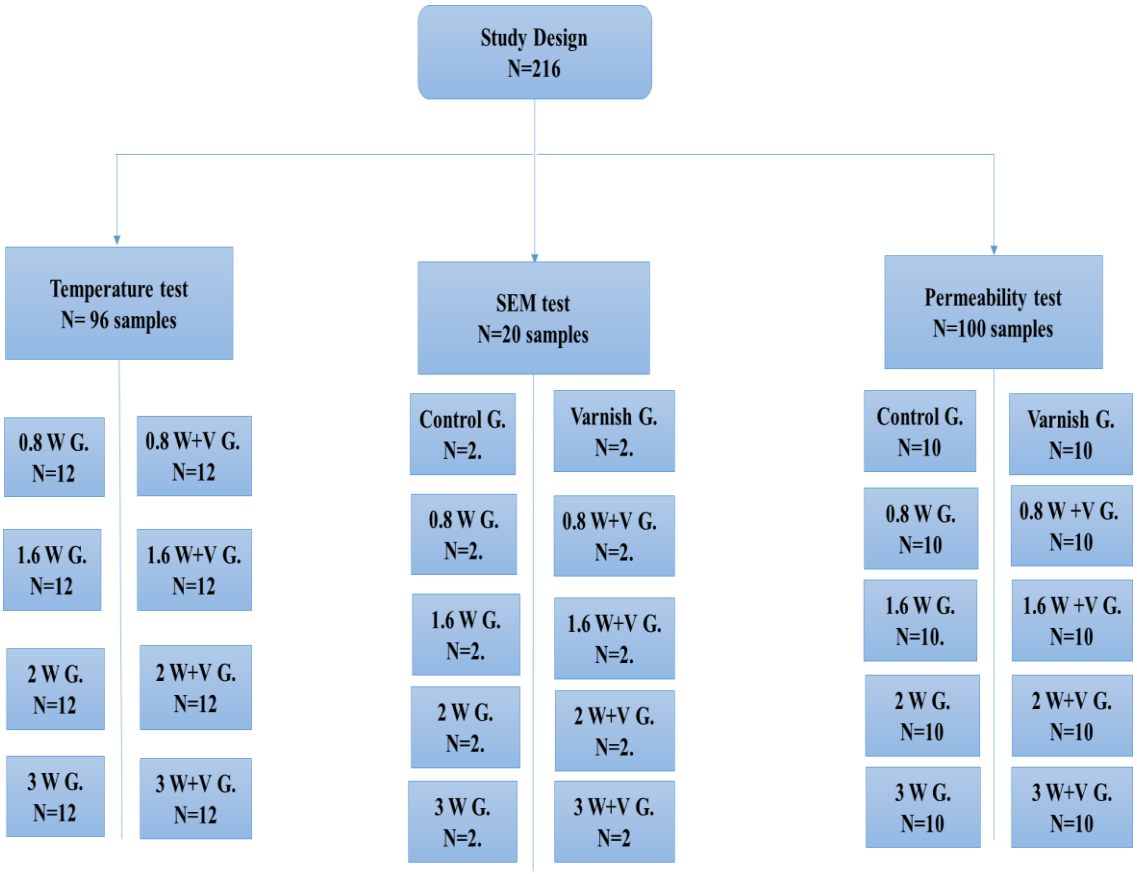


Figure 2-7: Study Design for the three investigations: Temperature measurement, permeability test and scanning electron microscope evaluation.

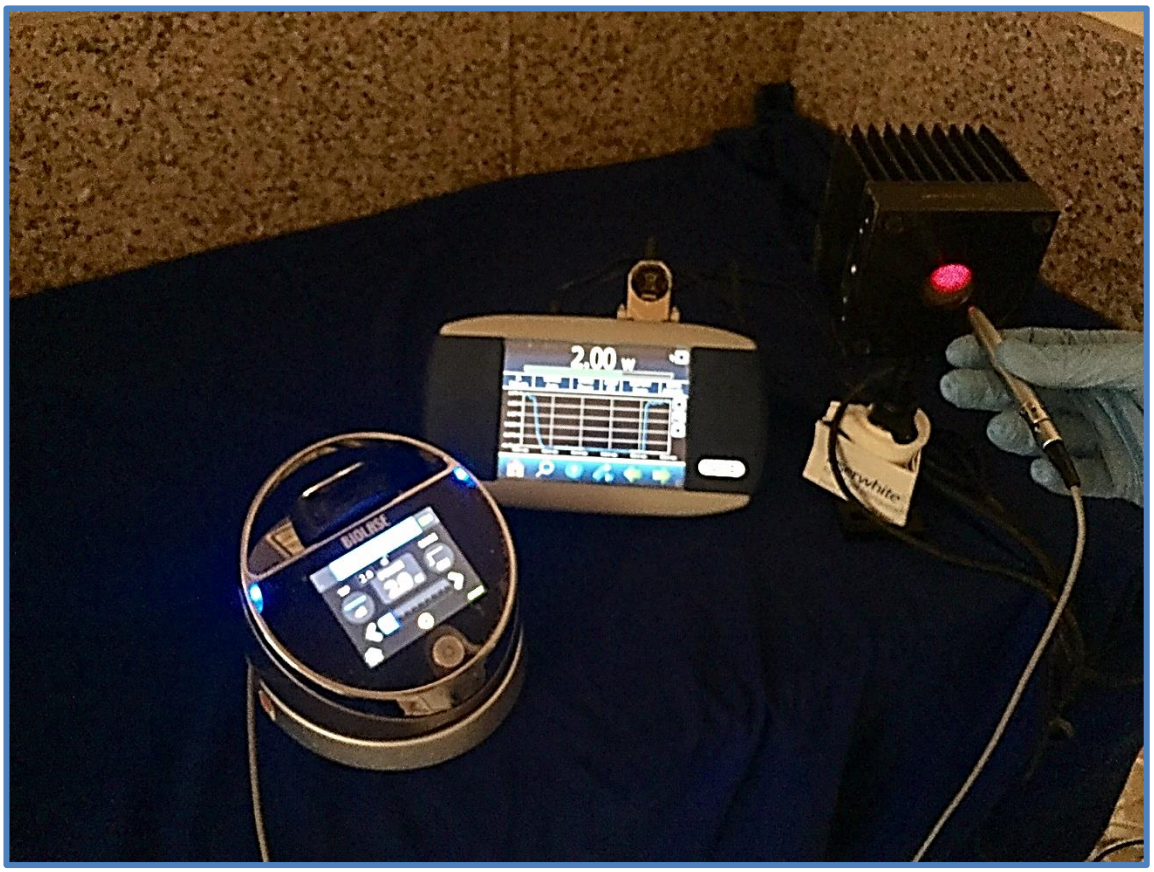


Figure 2-8: Diode laser 940nm calibration

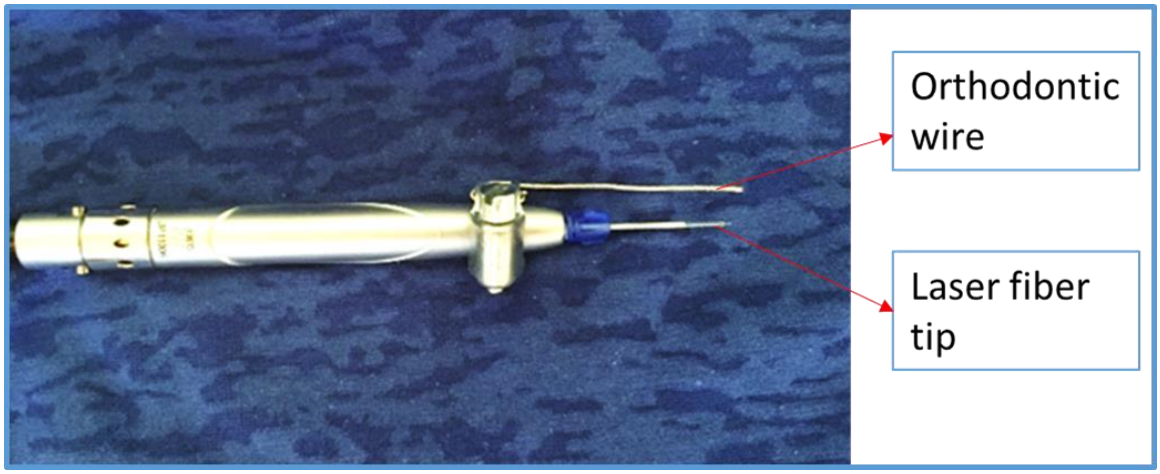


Figure 2-9: Orthodontic wire for distance fixation

2.2.3 Samples preparation

2.2.3.1 Samples preparation for temperature and thickness measurements

For temperature measurement 96 premolars were used (80 for pulp chamber temperature, 16 for external surface temperature). Teeth were

mounted on a holder for stabilization. The cementum was denuded with periodontal curette by 70 times strokes as stated by Coldiron et al.(95) with a periodontal curette by a gentle hand pressure in apico-coronal direction which was replaced every ten samples (Figure2-10, 2-11), then teeth were immersed in 17% EDTA solution for 1 minute (according to manufacturer instruction) for smear layer removal, then washed with distilled water in the ultrasonic cleaner for 15 minutes and dried with a 5 sec air blast. An area of 12 mm^2 (3X4 mm) in the buccal surface of the root cervical third was marked to determine laser irradiation area.

For pulp chamber temperature measurement, after teeth were remounted on a holder for teeth stabilization, a hole is made in the palatal surface of the root opposite to the laser irradiation area using diamond fissure bur at high speed turbine (300,000 rpm) under distilled water till reaching the pulp cavity for thermocouple insertion. (Figure.2-12).

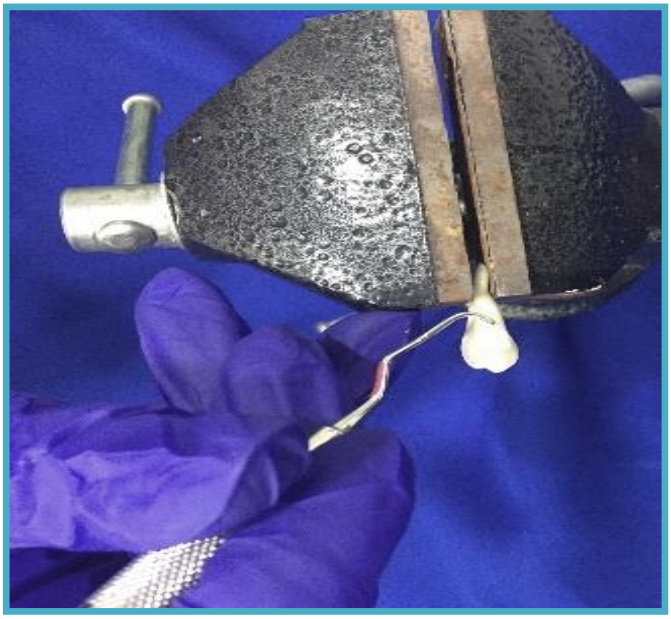


Figure 2-10: Cementum denudation with periodontal curette.

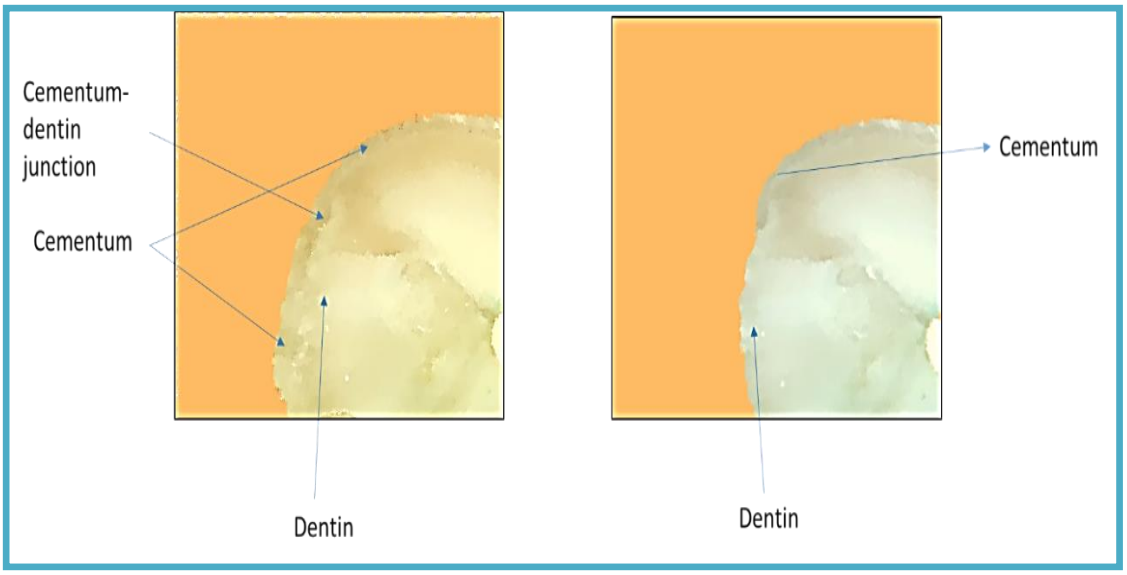


Figure 2-11: Microscopic views of a premolar tooth before and after cementum denudation from the buccal surface with 70 times curette strokes. Magnification x40.

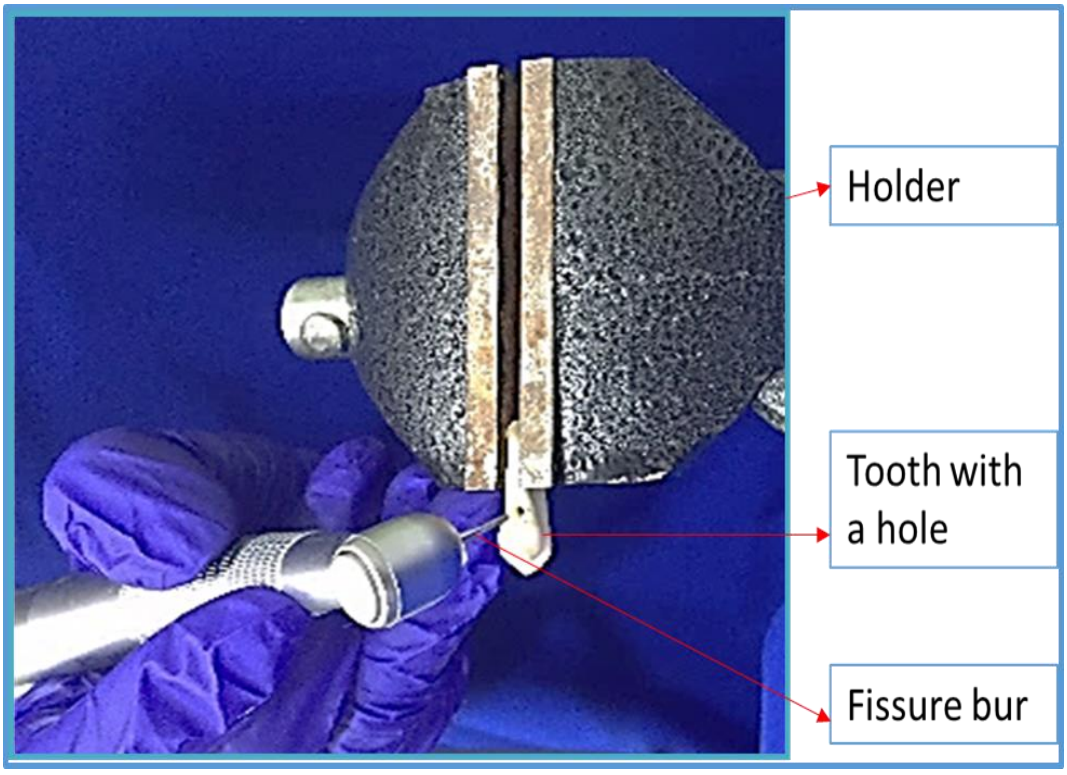


Figure 2-12: A hole is made in the palatal surface of the root opposite to the laser irradiation area by diamond fissure bur

2.2.3.2 Samples preparation for permeability test

A hundred teeth were used for permeability measurement. Samples were mounted on a holder for stabilization. After cementum was removed with a periodontal curette, two horizontal sectioning were made using a diamond disk mounted on low speed hand piece (250 rpm) under running distilled water. First section was done at the cement-enamel junction and second one was done 3 mm apical to the first one (Figure 2-13).

The specimens were immersed in 17% EDTA solution for 1 minute for smear layer removal, then washed with distilled water in the ultrasonic cleaner for 15 minutes and dried with a 5 sec air blast.

After marking an area of 12 mm² (3X4 mm) on specimen buccal surface to determine the area for laser irradiation, the specimens were coated with three coats of nail varnish except the marked area, then were mounted on a holder for specimen stabilization during laser irradiation (Figure.2-14).

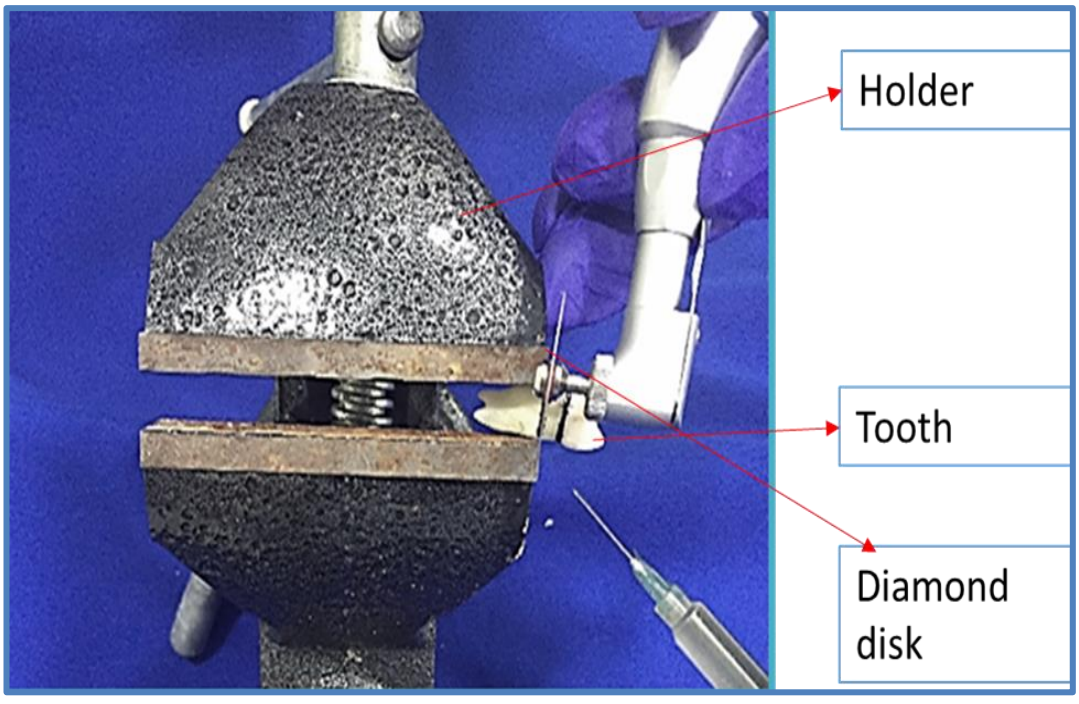


Figure 2-13: Two horizontal sectioning were made using a diamond disk mounted on low speed hand piece

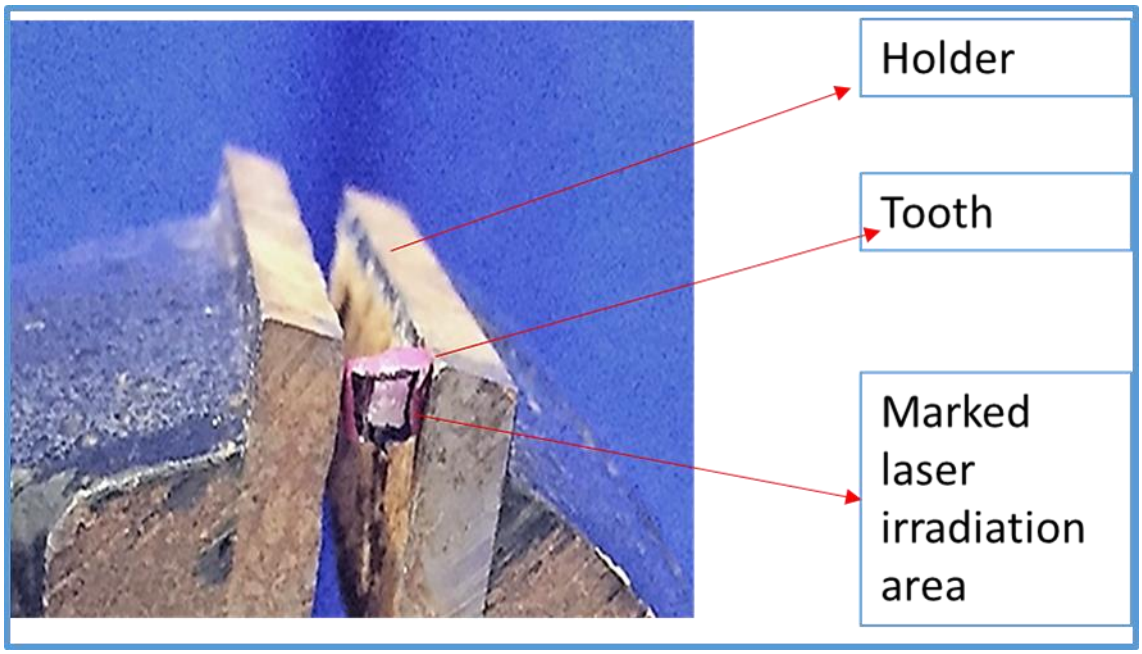


Figure 2-14: The samples were stabilize by a holder for laser irradiation

2.2.3.3 Samples Preparation for Scanning Electron Microscope (SEM)

Twenty premolar teeth were used for SEM analysis. Samples were mounted on a holder. After that, cementum was denuded with periodontal curette, then teeth were immersed in 17% EDTA solution for smear layer removal, then washed with distilled water in the ultrasonic cleaner and dried with a 5 sec air blast. To determine laser irradiation area, a twelve millimeters area (3X4 mm) in the buccal surface of the cervical third was marked.

A whole tooth was used for SEM test. The surface morphology of the samples were evaluated using SEM (Inspect S50).

2.2.4. Sodium Fluoride white varnish with TCP preparation and application

Varnish was thoroughly mixed, then two coats applied to the samples surface on the marked lasing area with its brush and allowed to set for 2 minutes in the presence of saliva according to manufacturer instruction. The artificial saliva was prepared following the protocol used by Karlinsey

et al(38). In 2 liters of distilled water, 0.7084 g calcium nitrate was added. After complete dissolution, 0.2450 g potassium phosphate monobasic was added. After complete dissolution, 19.383g potassium chloride was added. After complete dissolution, 8.56 g cacodylic acid was slowly added and allowed to mix for at least 15 minutes. Slow addition of concentrated HCl was added to adjust the pH to 7.0 by the aid of PH meter. The resulting solution then contained 1.25 mM Ca2+, 5.4 mM PO4, 20.4 mM K+, 24.5 mM Cl- and 6.5 mM Na+.

After samples were irradiated with laser at different used parameters, they were immersed in artificial saliva for 24 h to ensure complete treatment period according to manufacture instructions. Then each surface area, treated with varnish, was brushed manually with soft brush using modified bass technique (96), with 10 strokes for 10 seconds in oblique manner, in apico-coronal direction with gentle hand pressure to simulate patient teeth brushing (Figure 2-15).

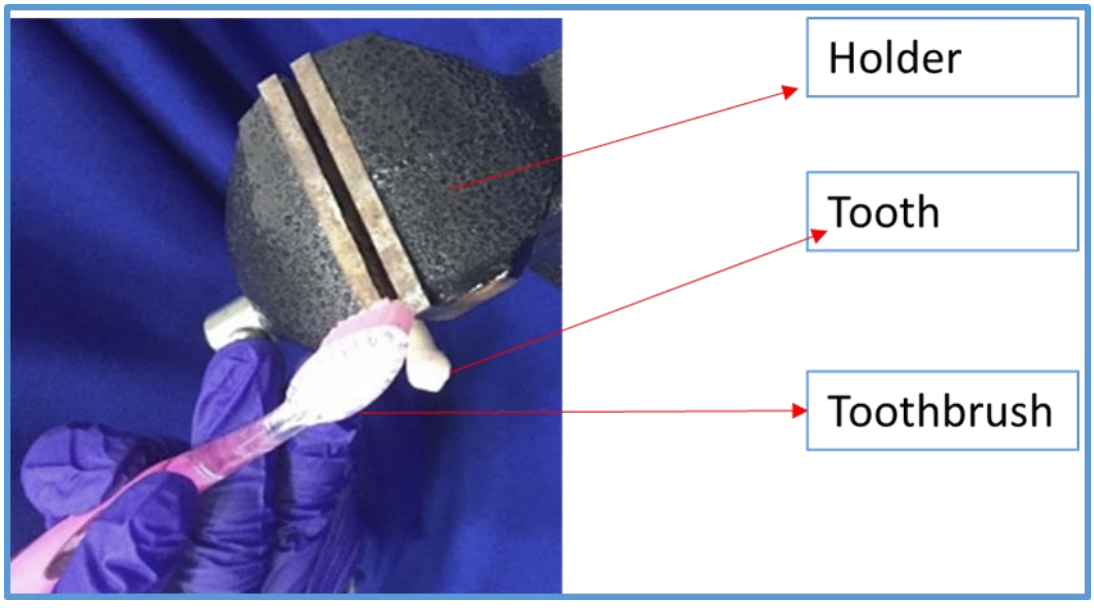


Figure 2-15: Each treated surface area with varnish was brushed manually with a soft brush in oblique manner, in apico-coronal direction.

2.2.5 Tests and measurements

2.2.5.1 Temperature and thickness measurements

2.2.5.1.1 Pulp chamber temperature measurement

For pulp chamber temperature measurement, the samples were stabilized with a holder (figure 2-16), then put inside a waterbath, in a way that part of the root immersed in water, which was heated to ensure 37 ± 0.5°C steady tooth temperature. Then K-type thermocouple placed in the pulp chamber through the predrilled hole opposite to the irradiation area. A thermal grease of 1 W/mK thermal conductivity was injected inside the pulp chamber, to verify contact between the thermocouple and the inner dentin surface during temperature assessments (Figure 2-17).

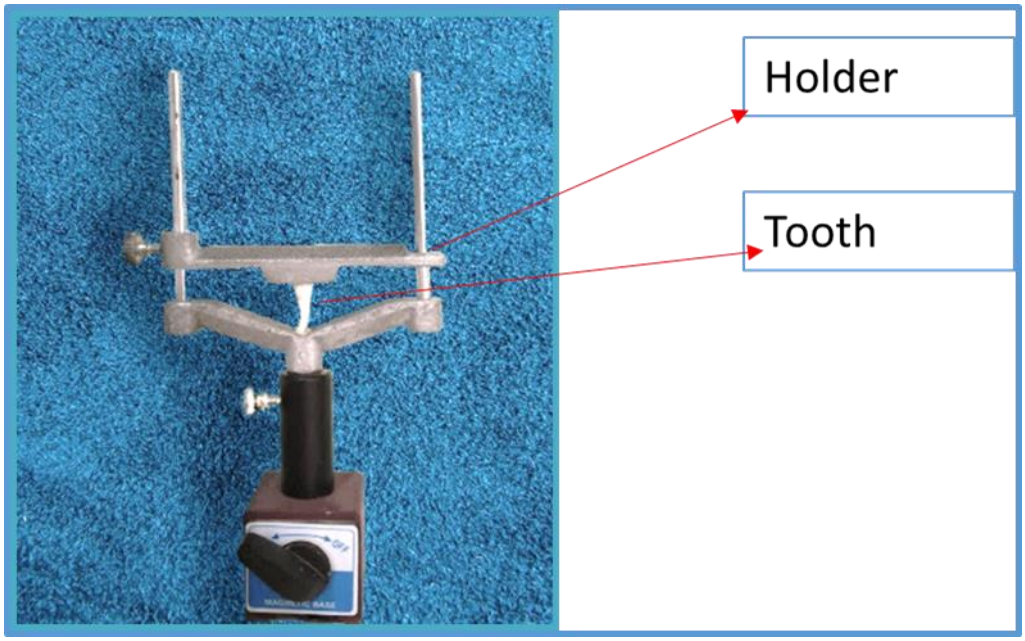


Figure 2-16: Sample stabilization on a holder

Temperature elevation during laser irradiation was measured using a K type thermocouple connected to a digital multilogger thermometer with basic accuracy of ± 0.05%, which was connected to a computer software program (AMPROBE multi-line V3.0) via a universal serial bus controller for data logging. The temperature recording was assessed every second (Figure 2-18).

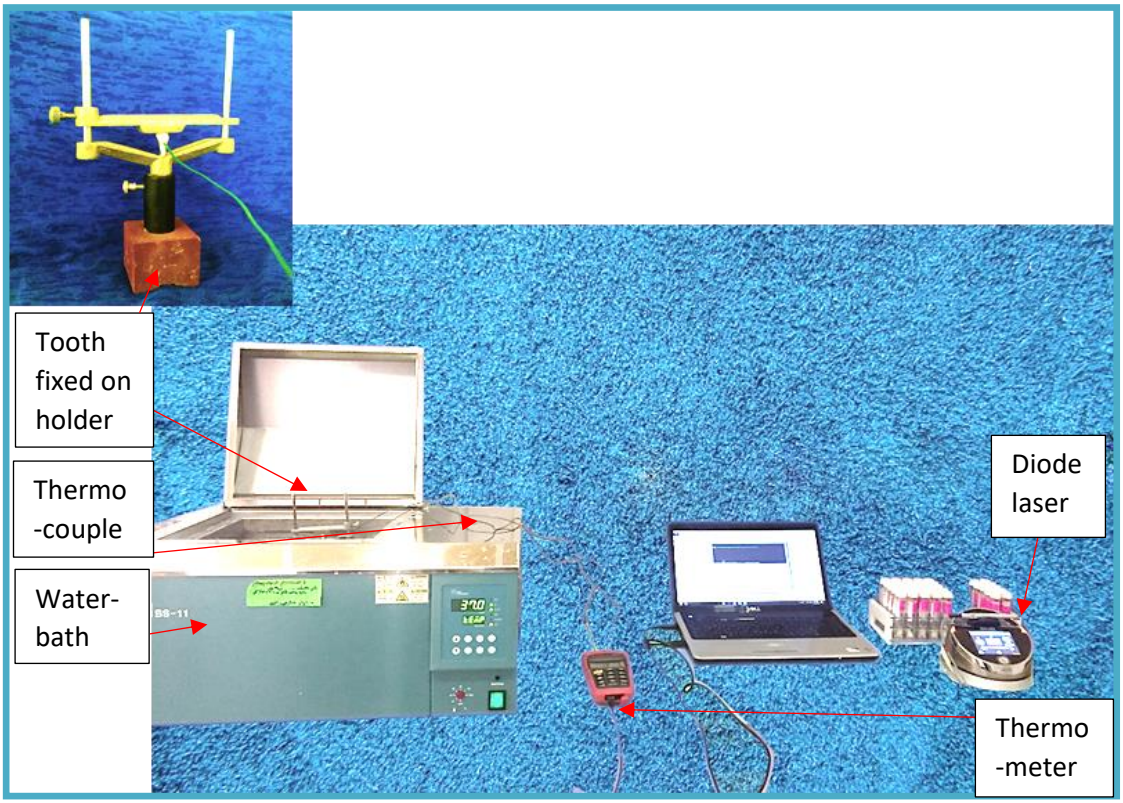


Figure 2-17: Pulp chamber temperature measurement experimental setup.

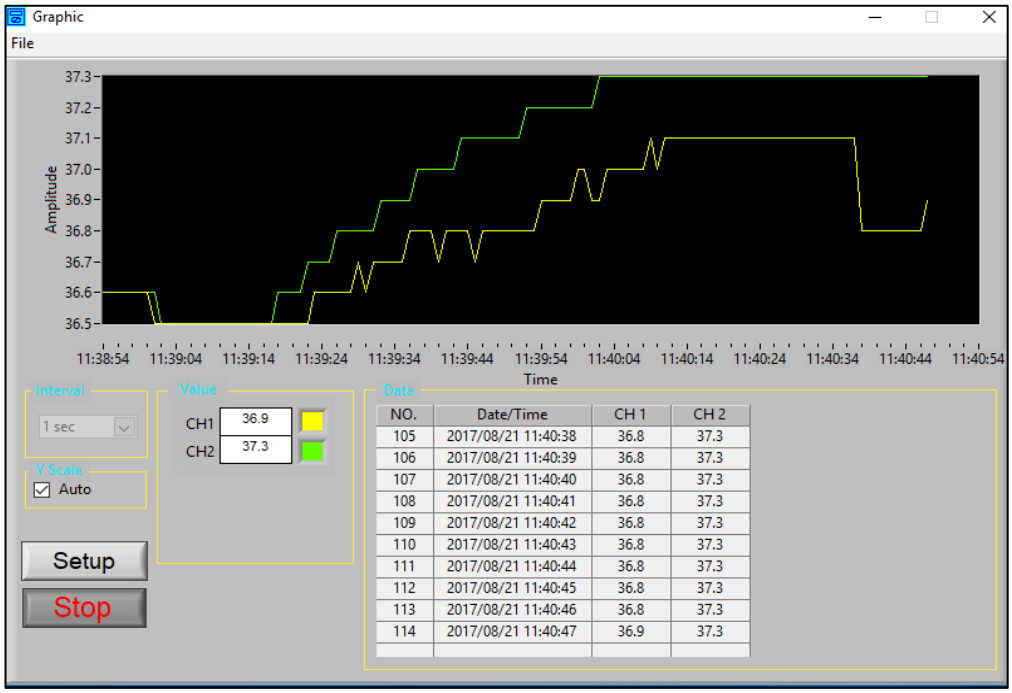


Figure 2-18: Amprobe program

2.2.5.1.2 Thickness measurements

After pulp chamber temperature measurement were obtained, the teeth were mounted on a holder and sectioned horizontally with a diamond disk through the mid-length of the marked lasing area, through the center of the predrilled hole, using a diamond disk mounted on low speed hand piece (250 rpm) under running distilled water. (Figure 2-19). After that, the dentin thickness is measured with the vernier caliper (Figure 2-20)

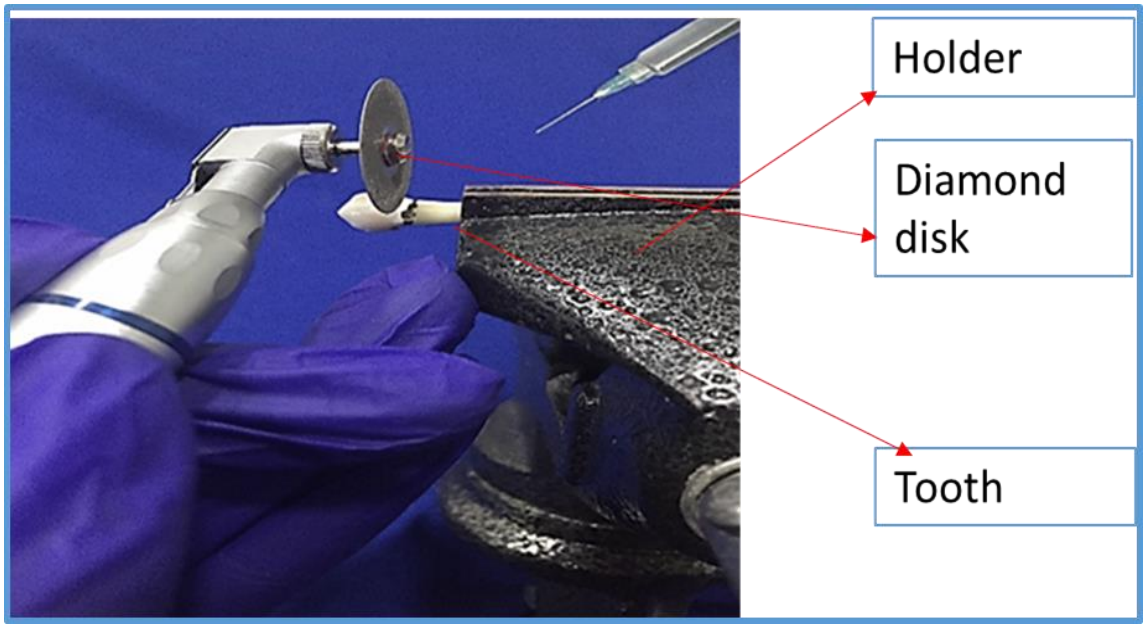


Figure 2-19: The teeth mounted on a holder and sectioned horizontally with a diamond disk through the center of the predrilled hole for thickness measurement.

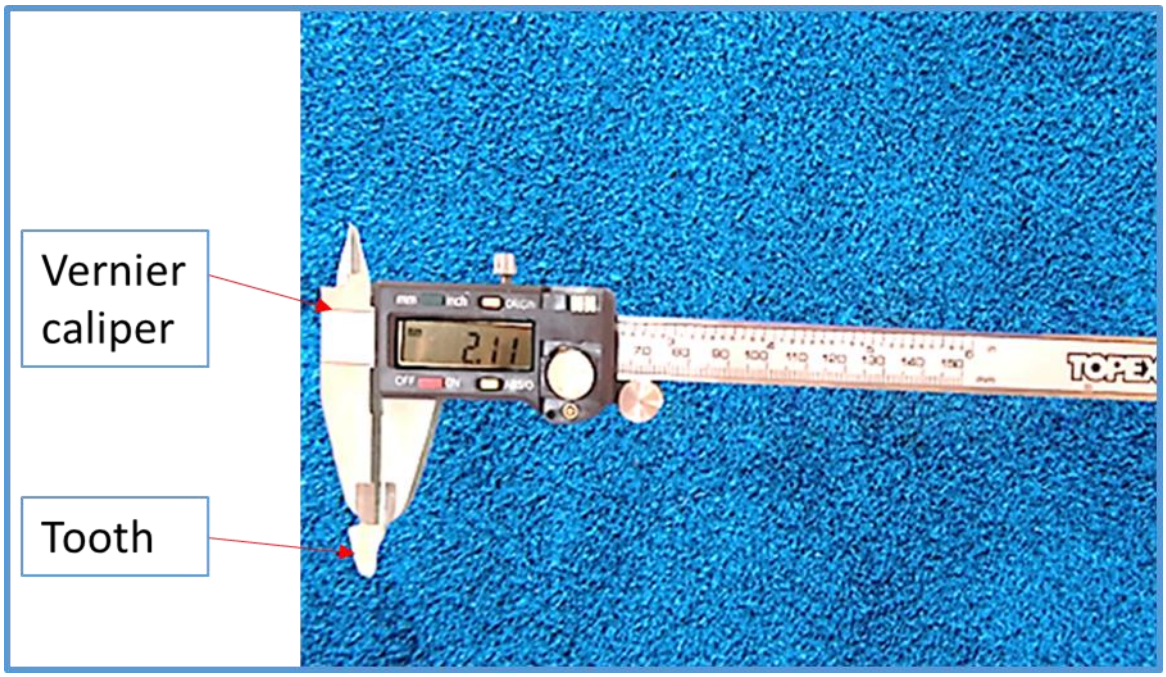


Figure 2-20 Measurement of the dentin thickness with the vernier caliper

2.2.5.1.3 External surface temperature measurement

For external surface temperature assessment, samples were mounted on a holder for stabilization during laser irradiation. A thermal camera (Flir i5) was positioned at 10 cm away from the sample as recommended by manufacturer, and a thermal image was taken during laser irradiation to measure temperature elevation of the external tooth surface (Figure 2-21).

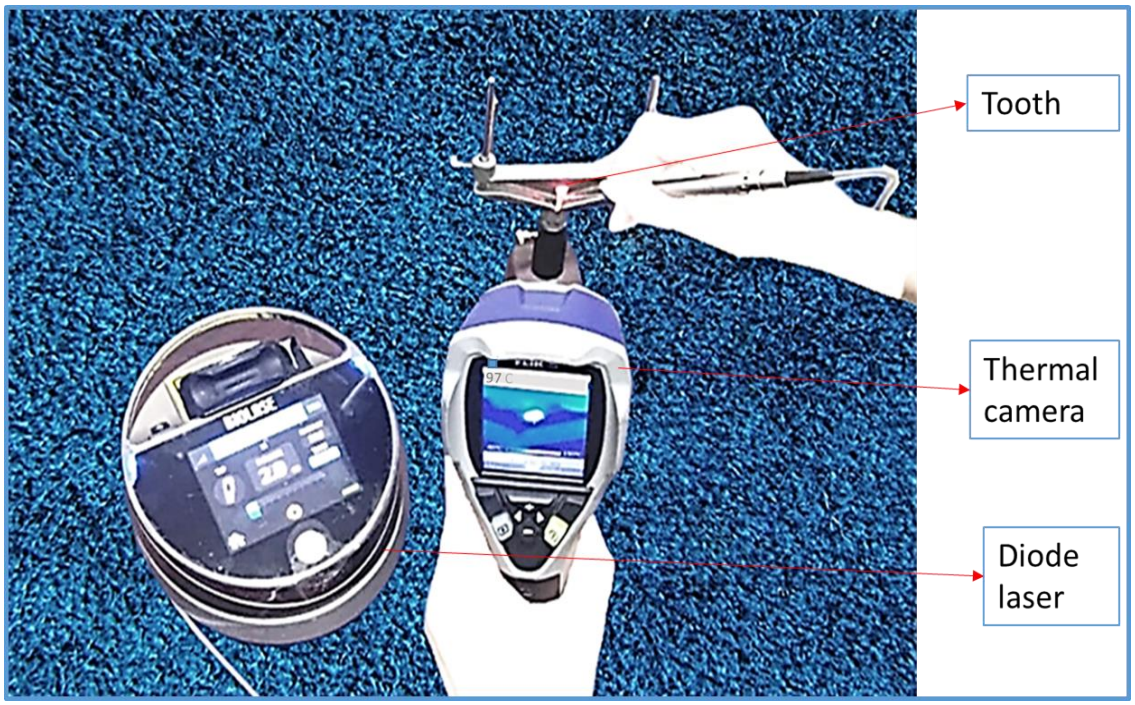


Figure 2-21: Setup of external surface temperature assessment

2.2.5.2 SEM test

Samples were mounted on a holder for stabilization during laser irradiation. After lasing, samples fixation and dehydration, in an ascending ethanol series, were done following the protocol used by Marchesan et al (97). The samples were placed in tubes with 2.5% buffered glutaraldehyde and 0.1 M sodium cacodylate at 4°C for 12 h, washed with distilled water for 3 min, immersed in distilled water for 1 h (the water was changed every 20 min), and then dehydrated in an ascending ethanol series: 25% (20 min), 50% (20 min), 75% (20 min), 95% (30 min), and 100% (60 min).

After dehydration, the specimens were left to dry for 24h, then fixed on aluminum stub and metallized with a layer of gold, using vacuum evaporation (Figure 2-22). The samples were analyzed by SEM (Inspect S50) with 2000x (50µm), 5000x (20µm) magnification, at 20 KV.

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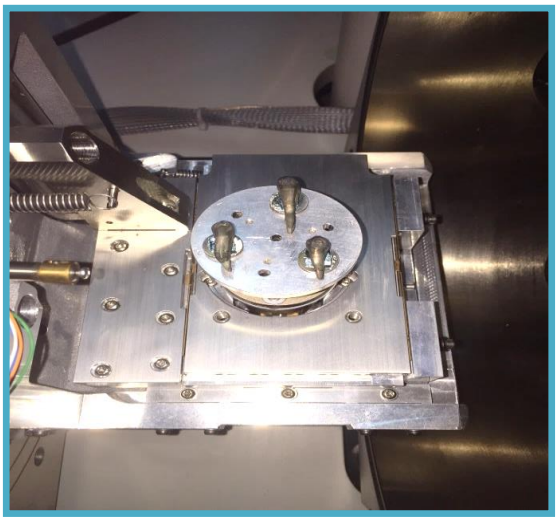


Figure 2-22: Teeth fixation on aluminum stub and metallization with a layer of gold.

2.2.5.3 Permeability Test

After samples preparation for permeability measurement, the samples were immersed in 2 % methylene blue dye for one hour at room temperature. Then, the samples were washed under tab water for 1 minute and left to dry, according to Al-Maliky et al protocol (72)

The samples were stabilize by a holder. Then, samples were sectioned using a diamond disk in the occlusal-apical direction in the midpoint of mesio-distal width using a diamond disk mounted on low speed hand piece (250 rpm) under running distilled water (Figure 2-23), the disk was replaced every ten samples.

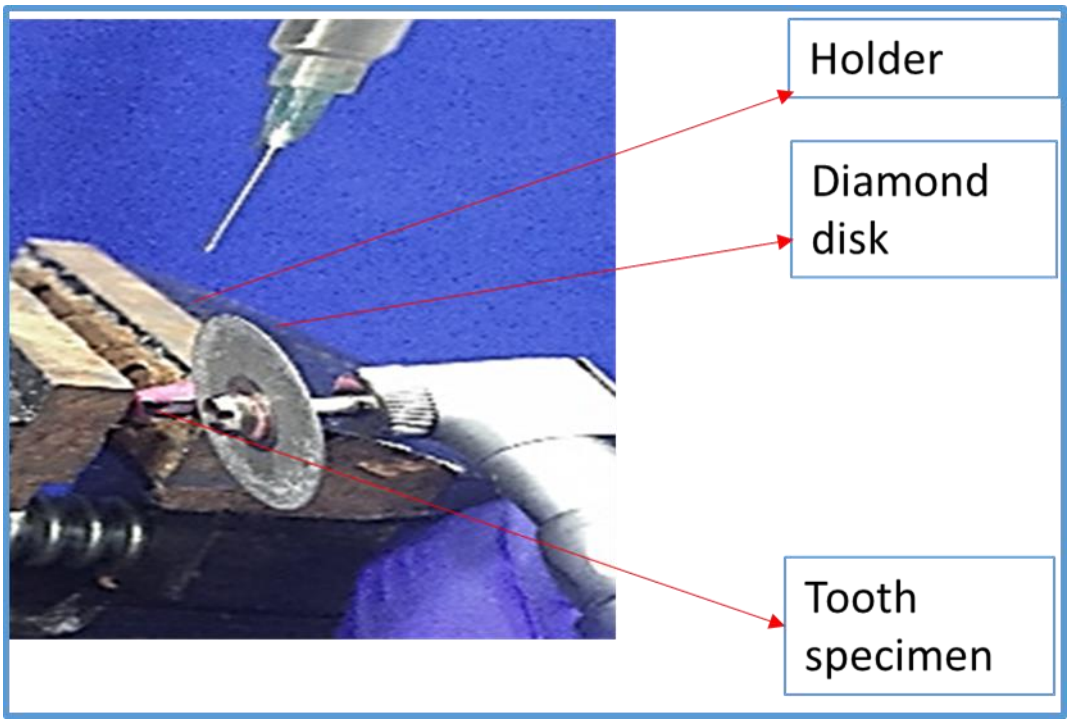


Figure 2-23: Samples sectioning using a diamond disk in the occlusal-apical direction in the midpoint of mesio-distal width.

The resultant specimens were placed on a sheet wax and evaluated using a stereomicroscope under a magnification of X40. The examined specimen photo introduced into computer software KAD-KAS Measure pictures V 1.0 on Microsoft Windows 10 operation system (Figure 2-24).

For standardization of the samples measurements, the ratio of dye penetration, from the outer surface of the dentine toward the pulp chamber, was estimated as follow:

Dye penetration ratio =

$$\frac{\text{depth of dye penetration inside the dentin}}{\text{Whole dentin thickness of the sample}} \times 100$$

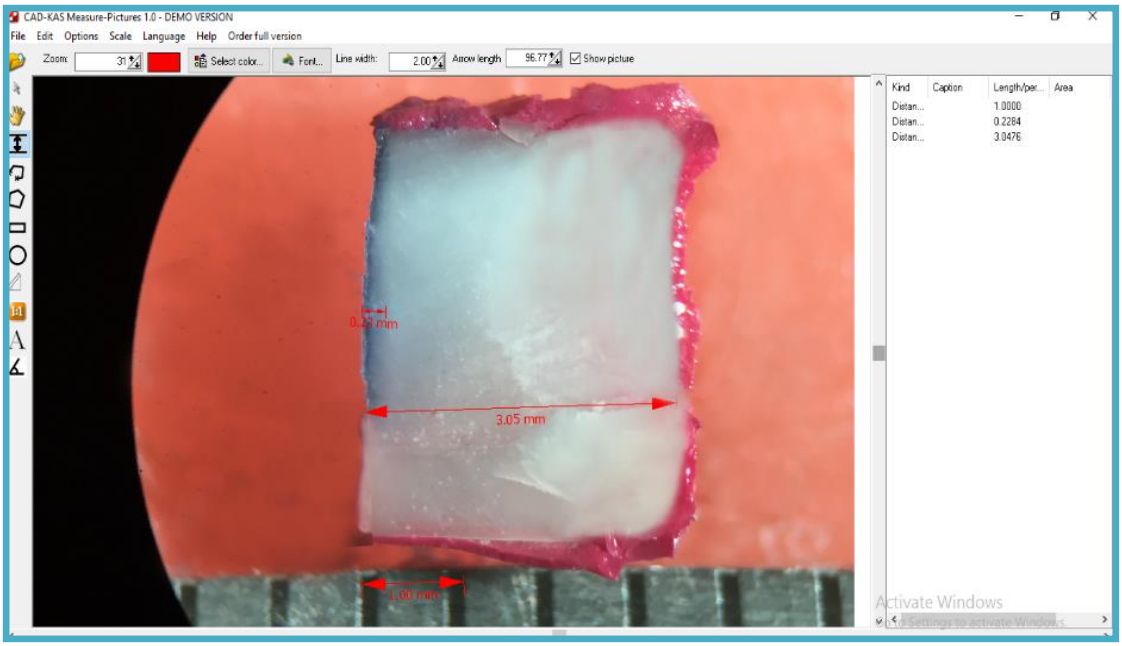


Figure 2-24: Dye penetration measurement using KAD-KAS program.

2.2.5.4 Absorption spectrum of varnish assessment

The absorption spectrum of varnish (Clinpro White Varnish) was determined with a Spectrophotometer calibrated in the spectral range from 900 to1000 nm. Two thin coating of varnish on a glass slide was analyzed, then absorbance calculated as follow:

Varnish absorbance = the resulted absorbance (varnish and glass) - glass slide absorbance

2.2.6 Statistical Analysis

All the results were statistically analyzed using SPSS V.23, and Excel 2010 for tables.

The statistical analysis consists of:

1- Descriptive Statistics:

• Means

• Standard deviations (SD)

• Standard errors (SE)

• Minimum values

• Maximum values

2- Inferential Statistics:

• **For pulp chamber temperature and thickness measurement**s

-For temperature measurement, Shapiro-Wilk test was done to estimate normality. After that Student's t-test was used to compare between treatments for each parameter.

-For thickness measurement, Pearson correlation test was done to investigate if there is a correlation between pulp chamber temperature elevation and dentin thickness measurement. After that Shapiro-Wilk test was used to investigate normal distribution of data. Then, in order to examine if the groups were statistically different, One-way ANOVA test was used.

• **For the permeability test**

To check if the acquired data had normal distribution, Shapiro-Wilk test was implemented. And to examine if the groups were statistically different, One-way ANOVA test was used. To examine hemogenousity of variance, Levene test was done. To investigate the difference between all groups, multiple comparisons were done using Dunette T test.

P > 0.05 **NS** (**N**ot **S**ignificant)

P ≤ 0.05 **S** (**S**ignificant)

P ≤ 0.01 **HS** (**H**ighly **S**ignificant)

Chapter Three
Result, Discussion and Future Work

Chapter Three

3.1 Results

This chapter includes the descriptive and inferential statistics of the quantitative evaluation of temperature and thickness measurements, and penetration ratios.

3.1.1Temperature and thickness measurements

3.1.1.1 Pulp chamber temperature record

Descriptive statistic for temperature measurement revealed that the maximum value of temperature change was 3 ˚C for 2W laser group. While the minimum value of temperature change was 0.2˚C for 0.8W+V group (Table3-1). The temperature rise for laser with varnish groups expressed less change compared to laser alone groups for each used power as shown in (figure 3-1).

Shapiro-Wilk test displayed normal distribution ($p>0.05$) (table 3-2) and Student- t-test for laser alone and with varnish groups for each used power showed no significant difference ($p>0.05$) (Table3-3).

Table 3-1: Descriptive statistics of temperature measurements (°C) among laser tested groups

Groups	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
0.8W	10	.7200	.25298	.08000	0.40	1.10
1.6W	10	1.6800	.93429	.29545	0.60	2.90
2W	10	1.8090	.81713	.25840	0.80	3.00
3W	10	1.5100	.54661	.17285	0.50	2.40
0.8W+V	10	.5600	.22211	.07024	0.20	1.00
1.6W+V	10	1.4700	.85641	.27082	0.40	2.70
2W+V	10	1.6600	.70427	.22271	0.80	2.80
3W+V	10	1.3800	.55538	.17563	0.50	2.10

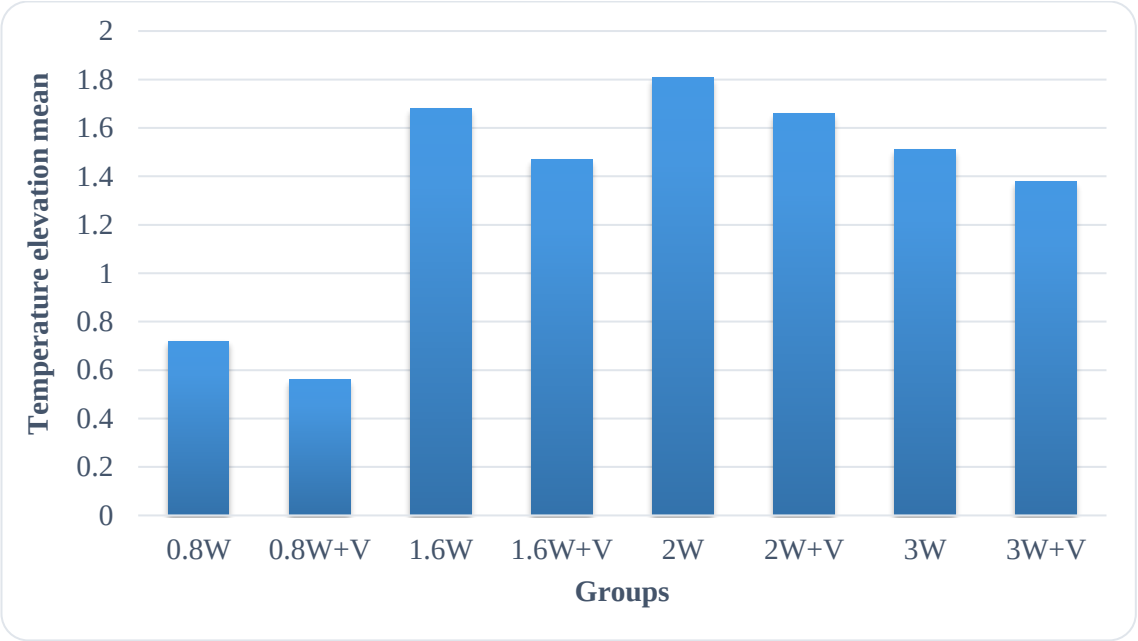


Figure3-1: Pulp temperature elevation mean differences among laser tested groups

Table 3-2: Shapiro-Wilk test for normality

Groups	Shapiro-Wilk		
	Statistic	N	Sig.
0.8W	.924	10	0.392
1.6W	.870	10	0.101
2W	.873	10	0.109
3W	.964	10	0.828
0.8W+V	.941	10	0.569
1.6W+V	.894	10	0.190
2W+V	.917	10	0.333
3W+V	.940	10	0.553

Table 3-3: Student-t-test for temperature measurement between laser alone and with varnish groups for each used power

Laser power	Varnish status				Statistics		
	With		Without		T	Sig.	
	Mean	±SD	Mean	±SD			
0.8W	.560	.222	.720	.253	1.503	0.150	NS
1.6W	1.470	.856	1.680	.934	0.524	0.607	NS
2W	1.660	.704	1.809	.817	0.437	0.667	NS
3W	1.380	.555	1.510	.547	0.528	0.604	NS

3.1.1.2 Samples thickness measurement

Descriptive statistics for samples thickness are disclosed in (Table 3-4).Mean difference among tested groups are shown in (Figure 3-2). To investigate if there is a correlation between pulp chamber temperature elevation and dentin thickness, Pearson test was done. Result revealed there is a high correlation between them. (Table 3-5)

To test normality among laser alone and with varnish groups, Shapiro-Wilk test was done. Result showed normal distribution ($p>0.05$) (Table 3-6). While to investigate if there is difference between groups, One-Way ANOVA test was implemented. The resulted data revealed no significant difference among tested groups (table 3-7).

Table 3-4: Descriptive statistics of laser alone and with varnish groups samples thickness (mm).

Groups	Mean	±SD	SE	Minimum	Maximum
0.8W	1.873	.134	.042	1.68	2.08
1.6W	1.867	.213	.067	1.54	2.20
2W	1.830	.151	.048	1.60	2.10
3W	1.897	.154	.049	1.66	2.12
0.8W+V	1.868	.175	.055	1.64	2.13
1.6W+V	1.912	.133	.042	1.67	2.10
2W+V	1.868	.190	.060	1.62	2.18
3W+V	1.857	.169	.054	1.60	2.09

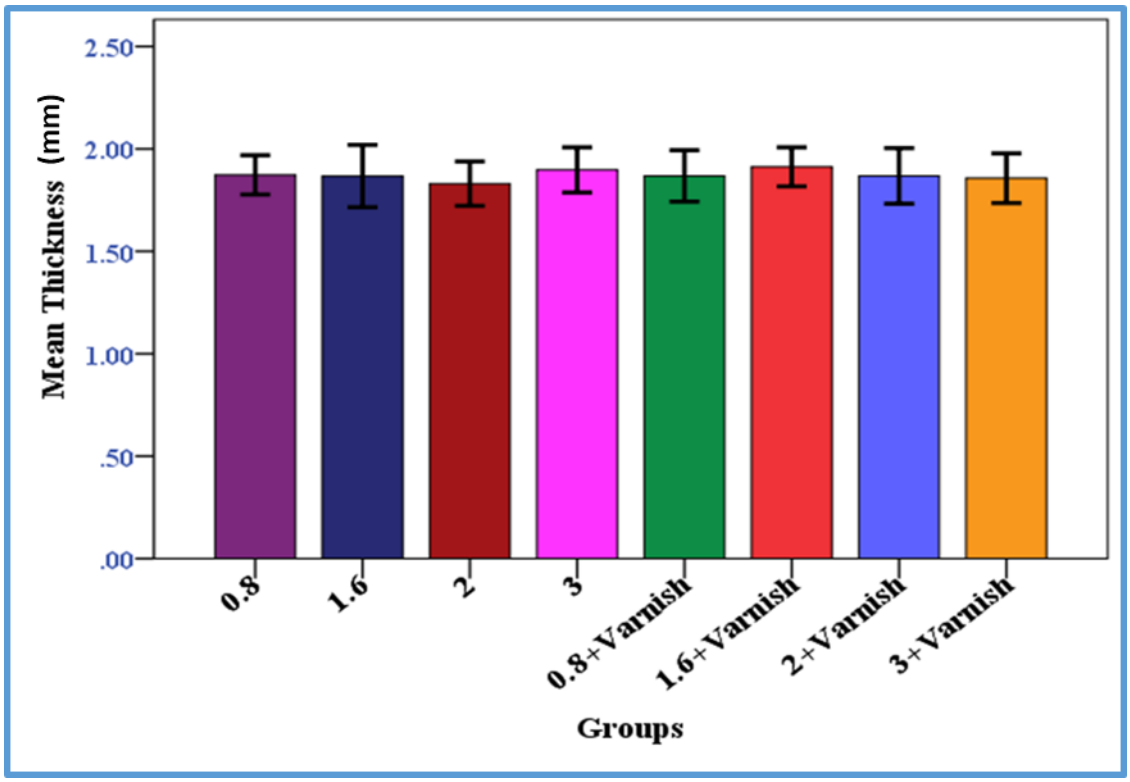


Figure 3-2: Thickness means difference (mm) among laser tested groups

Table 3-5: Pearson correlation test between pulp chamber temperature elevation and dentin thickness measurement (mm) among laser tested groups

Groups	Pearson correlation	Sig
0.8W	0.982	0.000
1.6W	0.964	
2W	0.937	
3W	0.961	
0.8W+V	0.918	
1.6 W+V	0.966	
2 W+V	0.935	
3 W+V	0.976	

Table 3-6: Shapiro-Wilk test for normality among laser alone and with varnish groups for thickness measurement (mm)

Groups	Shapiro-Wilk test			
	Stat.	N	Sig.	
0.8W	0.962	10	0.806	NS.
1.6W	0.973		0.921	
2W	0.987		0.992	
3W	0.948		0.648	
0.8W+V	0.933		0.482	
1.6W+V	0.910		0.278	
2W+V	0.947		0.632	
3W+V	0.946		0.625	

Table 3-7: One-Way ANOVA test among laser with and without varnish groups for thickness measurement (mm)

Groups	Statistics		
	F	df	Sig
0.8W	0.219	7	0.980 (NS)
1.6W			
2W			
3W			
0.8W+V			
1.6W+V			
2W+V			
3W+V			

3.1.1.3 Surface temperature measurements

Surface temperature assessment for laser without and with varnish groups as measured by thermal camera are shown in (table3-8). Data revealed that surface temperature range between 67-97.9°C for 1.6, 2 W and exceed 200 °C at 3W for both laser alone and with varnish groups.

Table3-8: Surface temperature measurements (°C) for laser without and with varnish groups

Groups	Sample (1)	Sample (2)	Mean
0.8W	49.9	51.5	50.7
1.6W	67	75.1	71.05
2W	90.4	83.6	87
3W	222	185.7	203.85
0.8W+V	58.5	61	59.75
1.6W+V	81.2	78.1	79.65
2W+V	97.9	95.6	96.75
3W+V	243	208.7	225.85

3.1.2 Permeability assessments

Descriptive statistics revealed the minimum recorded values were 0.42, 0.39 with group 2W+V and 3W+V group (Table 3-9). Penetration ratios mean difference among tested groups are shown in (Figure 3-3), the combined laser with varnish group showed less penetration compared to laser alone groups using the same parameter. Maximum dye penetration among tested groups was shown in (figures 3-[4-9]). To check if the acquired data had normal distribution, Shapiro-Wilk test was implemented, and the test statistics disclosed that data were normally distributed ($P > 0.05$) (see table3-10)

To examine if the groups were statistically different, One-way ANOVA test was used and the obtained descriptive level was (0.000), which revealed that the groups were highly significant different (see table 3-11)

To investigate the difference between each groups, after Leven test proved heterogeneous variances, multiple comparisons were done using Dunette T test. The result revealed that there is a significant difference between control group and 1.6W+V, 2W and 3W groups ($P< 0.05$), while the high significant difference occurred between control group and 2W+V, 3W+V groups ($P<0.01$) (see table 3-12).

Table 3-9: Descriptive statistics for dye penetration ratio among all tested groups

Groups	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Control	10	29.224200	12.1554757	3.8438989	9.5300	50.8000
Varnish	10	20.548000	14.6079558	4.6194412	2.8300	42.2000
0.8W	10	22.587000	13.8382209	4.3760297	2.0200	42.8000
0.8W+V	10	13.555000	10.8095064	3.4182660	1.8500	33.1000
1.6W	10	12.773000	7.9842304	2.5248353	1.2200	26.0000
1.6W+V	10	9.841100	7.9666027	2.5192610	2.2100	25.4000
2W	10	9.722000	7.4005973	2.3402743	.6000	23.1000
2W+V	10	7.694000	5.7734145	1.8257140	.4200	19.3000
3W	10	9.534000	6.9686858	2.2036920	.4600	20.6000
3W+V	10	7.632000	5.1942336	1.6425609	.3900	17.2000

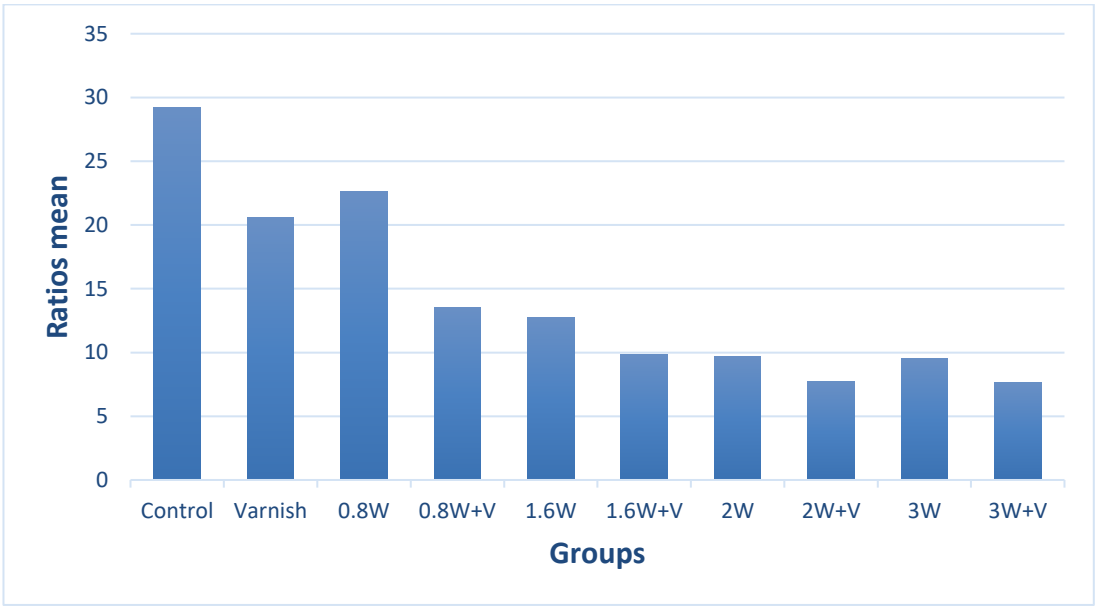
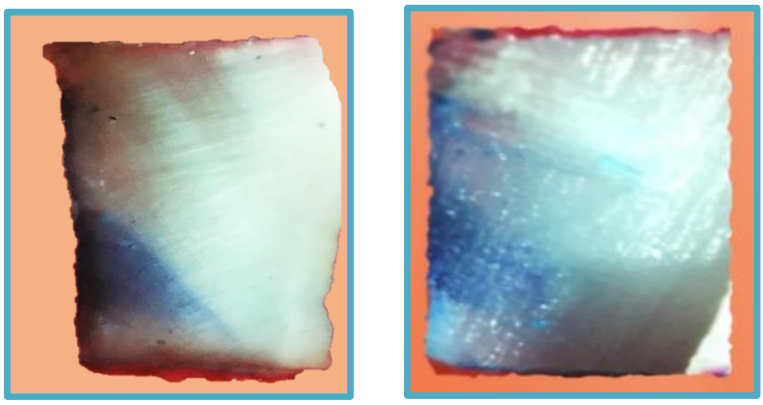
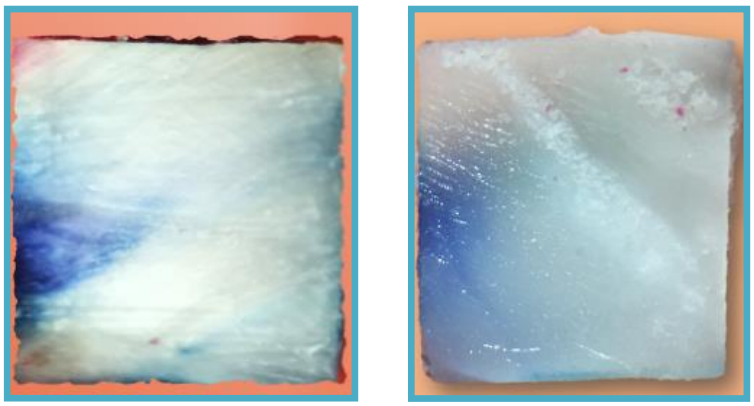


Figure 3-3: Penetration ratios mean difference among tested groups



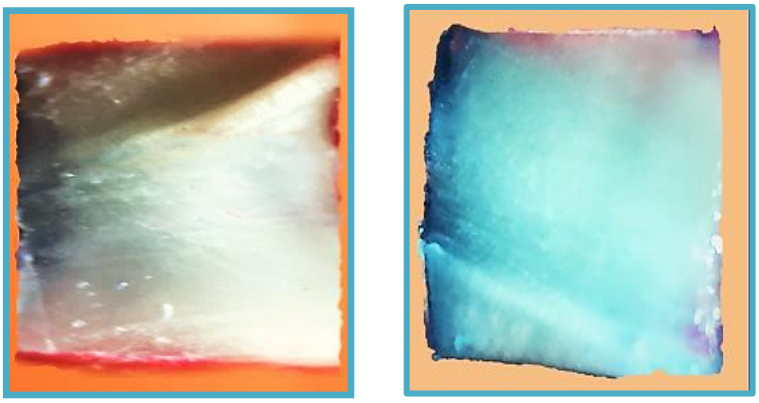
(a) **(b)**

Figure 3-4: Maximum dye penetration ratios among (a) control group, (b) varnish alone group under microscopic examination. Magnification X40.



(a) **(b)**

Figure 3-5: Maximum dye penetration ratios among (a) 0.8W group (b) 0.8W+Vgroup under microscopic examination. Magnification X40.



(a) **(b)**

Figure 3-6: Maximum dye penetration ratios among (a) 1.6W group (b) 1.6W+V group under microscopic examination. Magnification X40.

(a) (b)

Figure 3-7: Maximum dye penetration ratios among (a) 2W group (b) 2W+V group under microscopic examination. Magnification X40.

(a) (b)

Figure 3-8: Maximum dye penetration ratios among (a) 3W group, (b) 3W+V group under microscopic examination. Magnification X40.

Table3-10: Test of normality of dye penetration ratios among tested groups.

Groups	Shapiro-Wilk test			
	Statistic	N	Sig.	
Control	.974	10	.923	
Varnish	.901	10	.223	
0.8W	.960	10	.786	
0.8W+V	.894	10	.190	
1.6W	.933	10	.480	NS
1.6W+V	.845	10	.051	
2W	.912	10	.297	
2W+V	.918	10	.339	
3W	.938	10	.531	
3W+V	.964	10	.835	

Table3-11: One-Way ANOVA test for dye penetration ratios among all tested groups

Groups	Mean	SD	F	df	Sig.
Control	29.224	12.555	5.611	9	0.000 (HS)
Varnish	20.548	14.608			
0.8W	22.587	13.838			
0.8W+V	13.555	10.810			
1.6W	12.773	7.984			
1.6W+V	9.841	7.967			
2W	9.722	7.401			
2W+V	7.694	5.773			
3W	9.534	6.969			
3W+V	7.632	5.194			

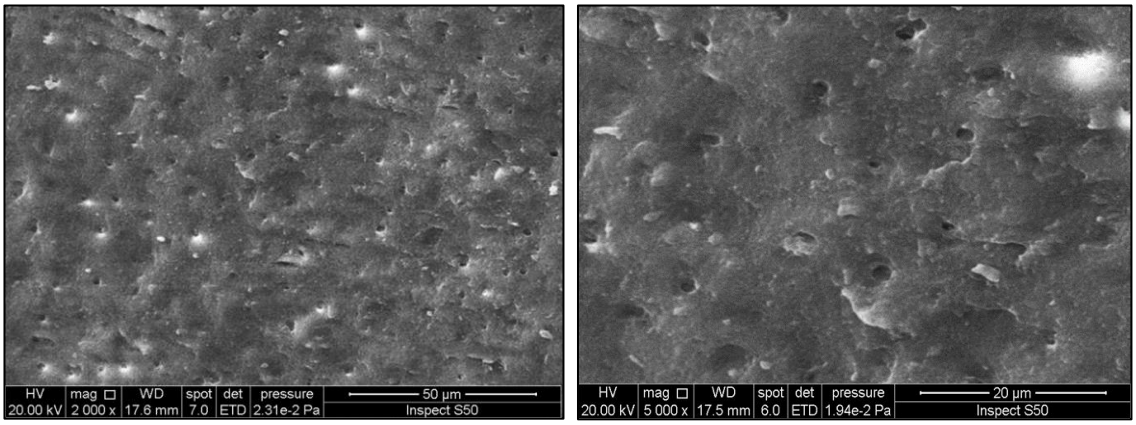
Table3-12: Dunette T test for dye penetration ratios comparison between all tested groups

Groups		Groups								
		Varnish	0.8W	0.8W+V	1.6W	1.6W+V	2W	2W+V	3W	3W+V
Control	MD	8.676	6.637	15.669	16.451	19.383	19.502	21.530	19.690	21.592
	Sig.	.991	1.000	.208	.086	.025*	.022*	.008**	.019*	.008**
Varnish	MD		-2.039	6.993	7.775	10.707	10.826	12.854	11.014	12.916
	Sig.		1.000	.999	.985	.795	.761	.460	.723	.437
0.8W	MD			9.032	9.814	12.746	12.865	14.893	13.053	14.955
	Sig.			.966	.846	.487	.448	.213	.410	.200
0.8W+V	MD				.782	3.714	3.833	5.861	4.021	5.923
	Sig.				1.000	1.000	1.000	.981	1.000	.972
1.6W	MD					2.932	3.051	5.079	3.239	5.141
	Sig.					1.000	1.000	.965	1.000	.944
1.6W+V	MD						.119	2.147	.307	2.209
	Sig.						1.000	1.000	1.000	1.000
2W	MD							2.028	.188	2.090
	Sig.							1.000	1.000	1.000
2W+V	MD								-1.840-	.062
	Sig.								1.000	1.000
3W	MD									1.902
	Sig.									1.000

(*) significant difference (**) highly significant difference

3.1.3 SEM Evaluation.

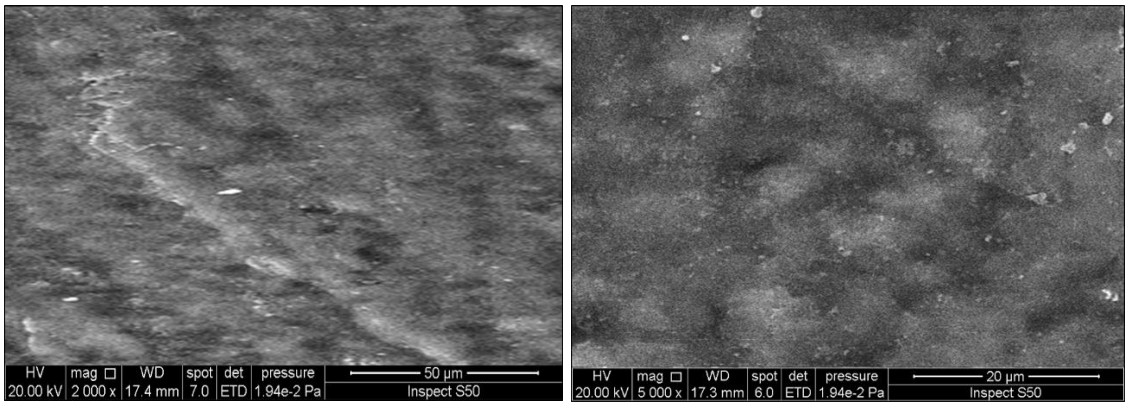
SEM analysis of the examined specimens showed surface structural changes. The control group presented open tubules and absence of the smear layer (figure3-9 (a) (b)).



(a) (b)

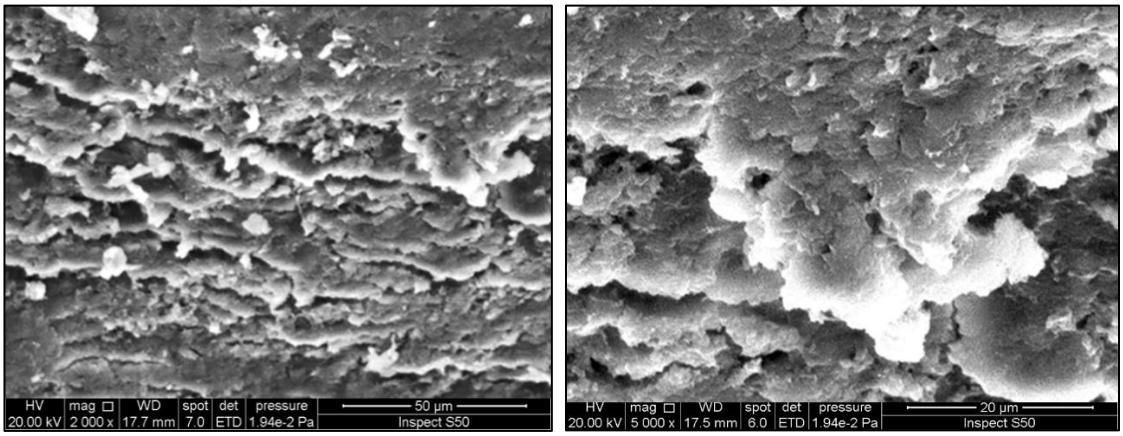
Figure 3-9: Scanning electron microscopic (SEM) views of (control group) treated only with EDTA (17%). The dentin is not covered by the smear layer, the tubules were open (a) (b) Magnification: 2000X, 5000X.

For the varnished alone group, first SEM views for varnish group without brushing to estimate immediate occlusion effect, then after brushing to simulate an effect of patient teeth brushing. Note nearly optimum seal of DTs with varnish without brushing (figure 3-10 (a) (b)), while after brushing only few tubules were occluded (figure 3-11 (a) (b)).



(a) (b)

Figure 3-10: Scanning electron microscopic (SEM) views of NaF white varnish group before brushing. Note nearly optimum seal of DTs with varnish without brushing (a) (b). Magnification: 2000x, 5000x.

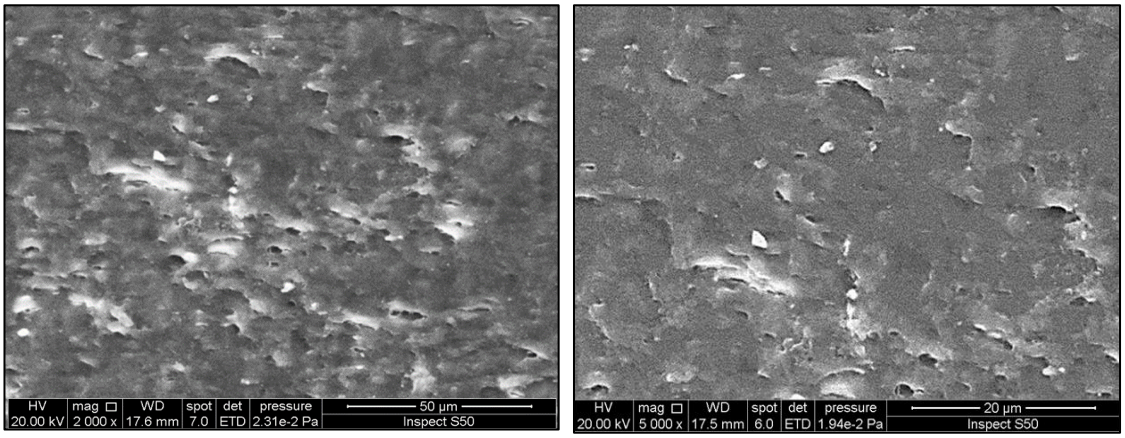


(a) (b)

Figure 3-11: Scanning electron microscopic (SEM) views of (varnish group) after brushing, only few tubules were occluded (a) (b). Magnification: 2000x, 5000x.

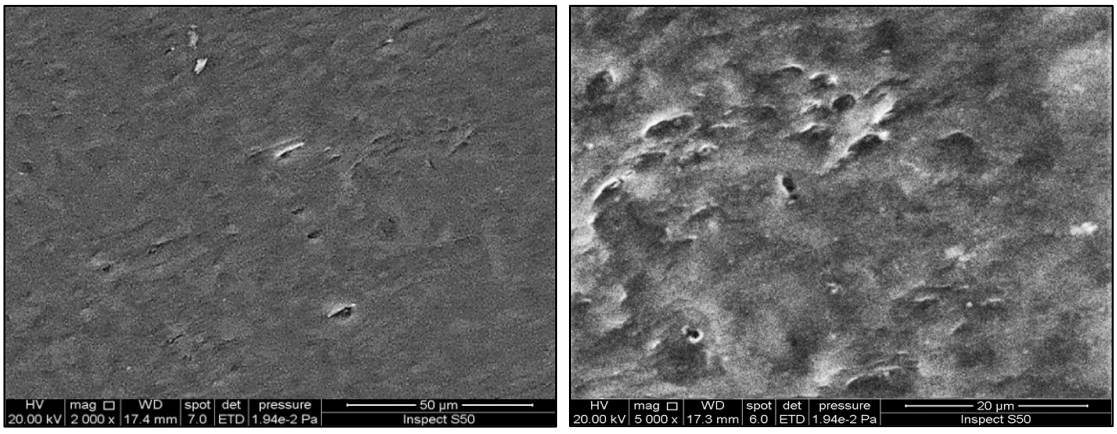
For the laser irradiated alone groups, only few narrowed dentinal tubules was observed at 0.8W group (figure 3-12 (a) (b), narrowed and few sealed tubules observed at 1.6W groups, (figure 3-13 (a) (b)). While for 2W group, several dentinal tubules were occluded due to protein denaturation without signs of cracking or char. (figure 3-14 (a) (b)). At 3W group, laser

irradiation provoked an amorphous form with darkened areas indicating dentin carbonization and destruction (figure 3-15(a) (b)).



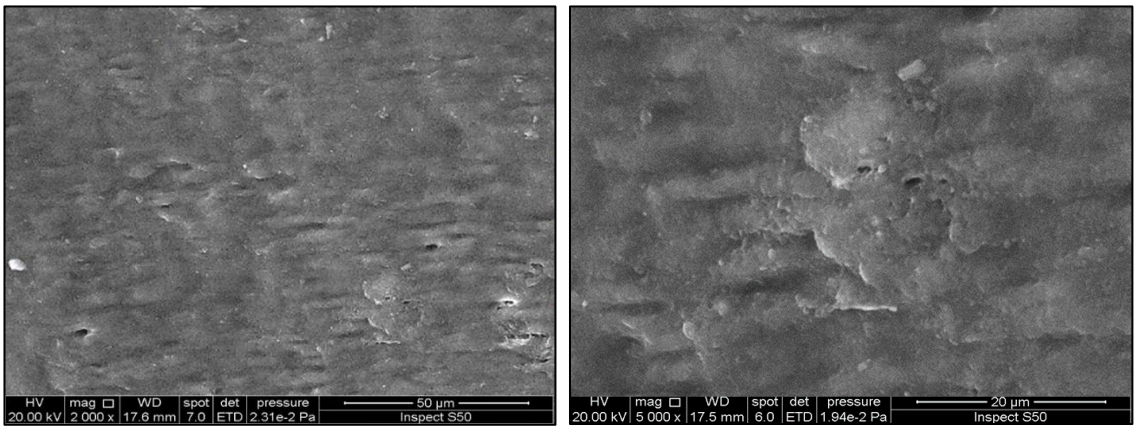
(a) (b)

Figure 3-12: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 0.8W. Only few narrowed dentinal tubules can be noted (a) (b). Magnification: 2000x, 5000x.



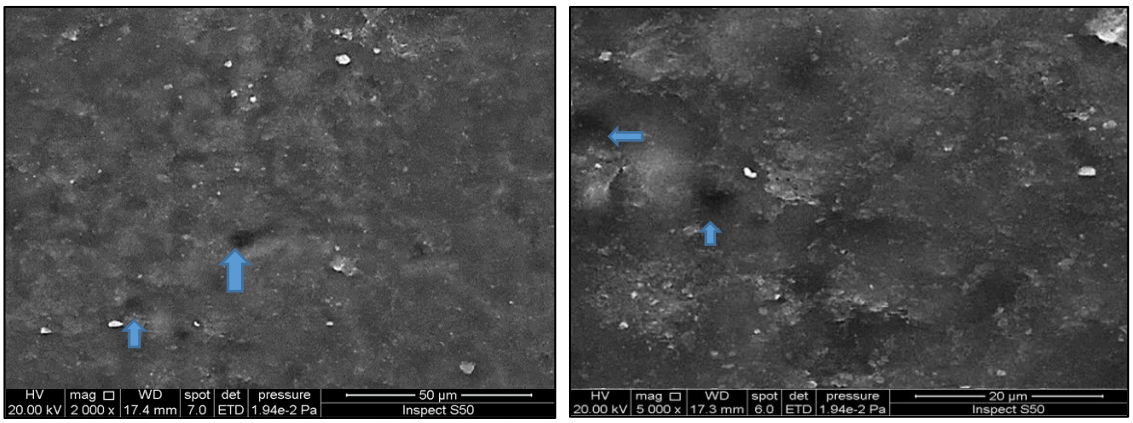
(a) (b)

Figure 3-13: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 1.6W. Narrowed and some occluded dentinal tubules can be noted (a) (b). Magnification: 2000x, 5000x.



(a) (b)

Figure 3-14: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 2W. Several dentinal tubules were sealed due to protein denaturation without signs of crack or char (a) (b). Magnification: 2000x, 5000x.

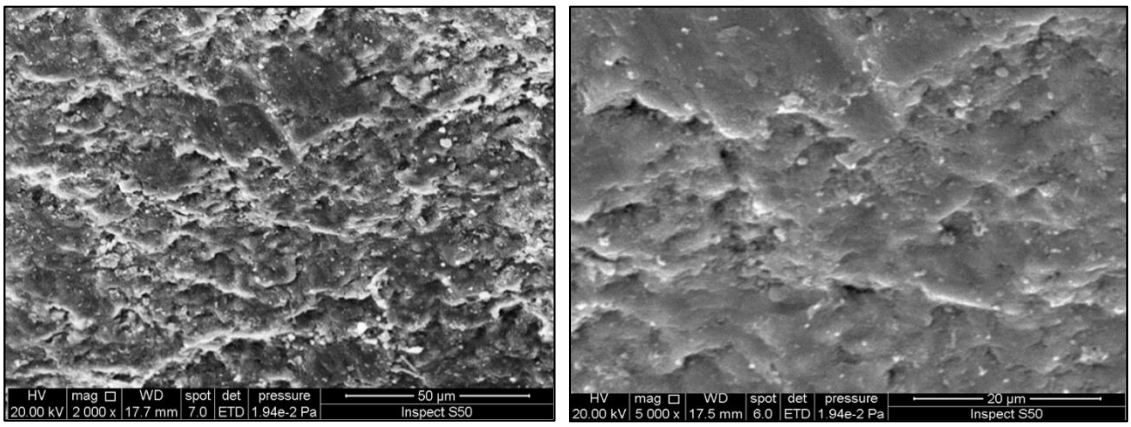


(a) (b)

Figure 3-15: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 3W+V. Some areas of dentin carbonization and destruction can be noticed (arrows) (a) (b). Magnification: 2000x, 5000x

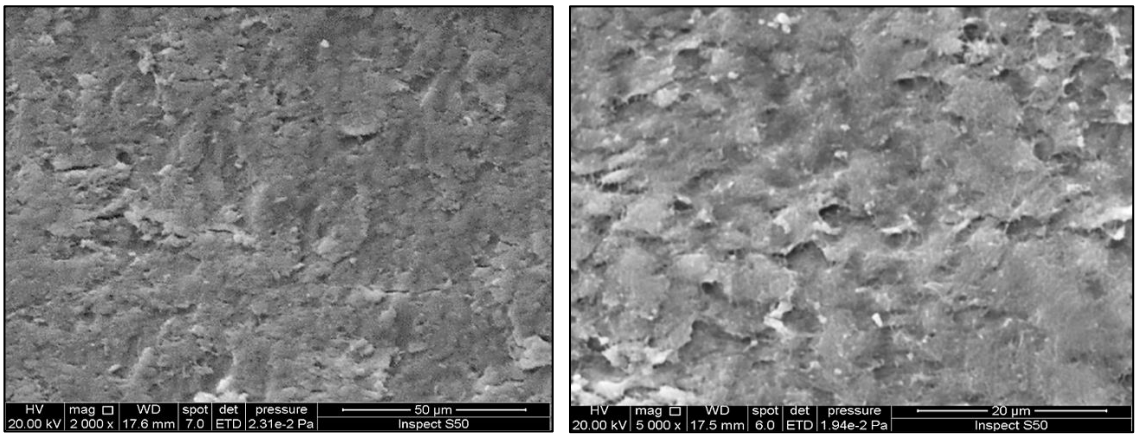
While in the combination groups (laser with varnish after brushing), more number of DTs were narrowed or occluded at 0.8W+V, 1.6W+V group compared to laser without varnish groups (figure 3-16 (a) (b), 3-17(a) (b). At 2W+V almost optimum sealing of tubules were observed without signs of carbonization or cracking (figure 3-18(a) (b). At higher

output power (3W), excessive surface destruction with carbonization track were noticed (Figure 3-19(a) (b)).



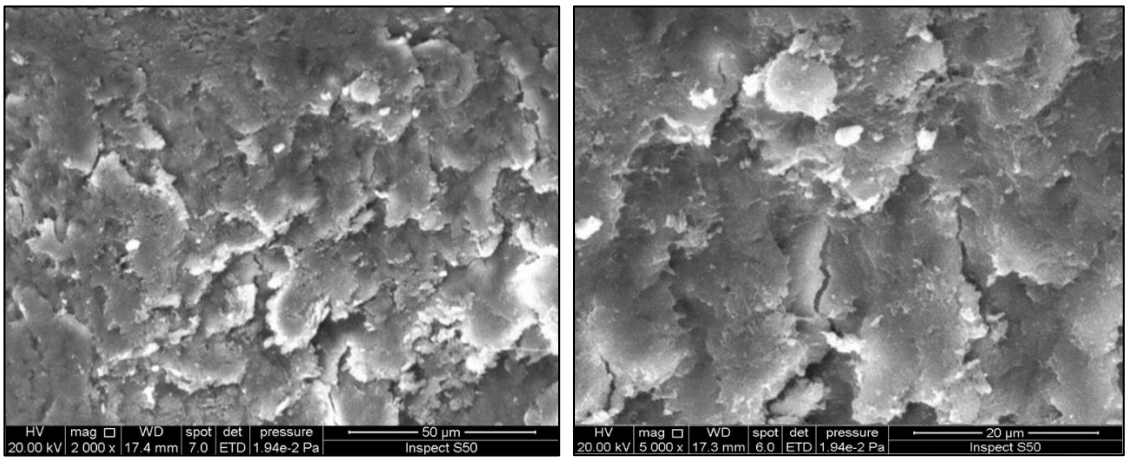
(a) (b)

Figure 3-16: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 0.8W with varnish. Narrowing and some occlusion of DTs were observed (a) (b). Magnification: 2000x, 5000x.



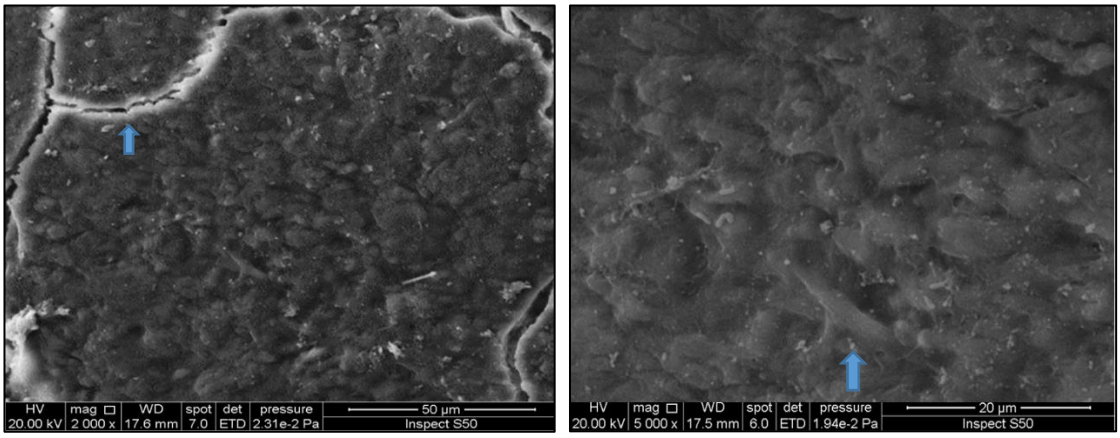
(a) (b)

Figure 3-17: Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 1.6W with varnish group. Several occluded DTs were observed (a) (b). Magnification: 2000x, 5000x.



(a) (b)

Figure 3-18: Scanning electron microscopic (SEM) views of treated dentin by the diode laser 2W with varnish. Almost an optimum sealing of DTs were observed without signs of carbonization or cracking. Magnification: 2000x, 5000x.



(a) (b)

Figure 3-19. Scanning electron microscopic (SEM) views of treated dentin by the diode laser at 3W with varnish, excessive surface destruction with carbonization track were noticed (arrows) (a) (b). Magnification: 2000x, 5000x.

3.1.4 NaF white varnish spectrum assessment

Data of absorption spectrum analysis for Clinpro white varnish in the range of (900-1000nm) was illustrated in (Figure 3-20).Data revealed that varnish absorbance for 940nm was 0.808, which was higher than absorbance for 980nm.

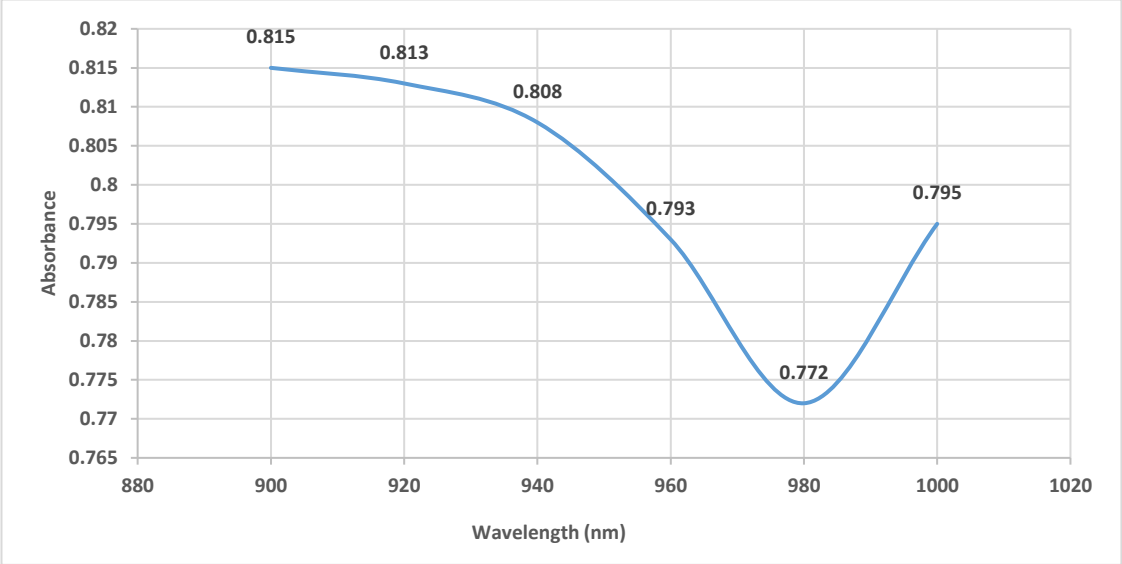


Figure 3-20: Clinpro white varnish absorption spectrum for (900-1000 nm)

3-2 Discussion

Dentin hypersensitivity is correlated to the number of exposed tubules. Fluid movement inside the exposed DTs due to various stimuli leads to pain sensation (98). DTs diameter reduction and blockage will prevent intratubular fluid movement and consequently will lead to DH pain relief. Hence the main aim of a successful hypersensitivity treatment is the partial or complete occlusion of dentinal tubules (99).

In this study, atrial was made to assess the ability of diode laser (940nm) laser alone or in combination with sodium fluoride white varnish with tri calcium phosphate to reduce diameter or close opened dentinal tubules, concerning effects on dentin morphology, permeability and temperature elevation since the type of tissue interaction in this experiment is photothermal.

As there is no standardization for the 940 nm diode laser in dentin hypersensitivity treatment, the experiment was done in-vitro to investigate the aspects of success and safe parameters to dental tissues before any in-vivo application.

A laser wavelength of between 800 and 980nm has a poor absorption in water and hydroxyapatite (52) with little absorption in dental tissues (by its mineral structures such as phosphate and carbonate) (85, 100). This low absorption prevails scattering, or diffused transmission of the laser radiation through the dentin, and important thermal effects (101).

In this research, continuous wave mode was used to ensure scanning the whole dentin surface, noncontact mode to protect the optical fiber from contamination with varnish and damage by the surface heat, in oblique manner to reduce a direct pulp exposure by the part of laser radiation that was not absorbed by dentin (85) or varnish.

3.2.1 Temperature and thickness measurements

3.2.1.1 Pulp chamber temperature measurement

Since the type of tissue interaction in this experiment is photothermal, it is important to ensure that the temperature changes due to the used laser parameters in this study are within the pulp safety threshold of 5.5 °C temperature increase(102).

The maximum recorded value of temperature rise for all used laser settings was 3 °C inside the pulp chamber, with an average dentin thickness of 1.871 mm. This indicates that the used laser settings were within the pulp safety limits.

The temperature results for the pulp chamber in our study are comparable with Umana et al. results for the same used power settings with 980 nm diode laser which is close to 940nm.(85)

If we added to that the body effect of cooling by circulation and heat dissipation, the used power settings would be safer to the pulp.

The additional absorption by varnish in the laser with varnish groups, as examined by spectrum absorption assessment (figure 3-20), may explain their lower pulp temperature increase compared with laser without varnish groups, since the penetrated energy to the pulp was decreased according to Beer-lambert law .

At high power 3W a drop in pulp temperature was noticed, which could be as a result of increased dentin surface absorption due to carbonization. Carbon acts as a heat sink as laser irradiation continues since it is high absorber of (800-1064 nm) wavelengths causes a dramatic degradation of the forward power transmission (103).

3.2.1.2 Dentin thickness measurement

The correlation between the remaining dentin thickness in the middle of lasing area and pulp chamber temperature rise was measured. It

showed a high correlation which was accepted with the basic law of thermodynamics.

Therefore a sample with a less dentin thickness (m) would have exhibited a higher temperature change (ΔT) after the lasing procedure. This may aid clinicians to choose the correct parameters according to the predicted tooth thickness to avoid thermal injury to the pulp.

However in this study, thickness assessment showed no significant difference in dentine thickness for laser with and without varnish groups' samples. This could eliminate the effect of dentine thickness on pulp temperature measurement for this study.

3.2.1.3 Surface temperature measurement

In order to assess which photothermal interaction was take place on the dentin surface related to different used laser parameters settings, surface temperature was measured using thermal camera.

The results revealed that the temperature rise was between (67-97.9 °C) for 1.6, 2 W laser with and without varnish groups that means protein denaturation of dentin organic components was occurred, and it exceed 200 °C at 3W for both laser alone and with varnish groups which indicate surface carbonization was occurred (45). Results were accepted with Trushkowsky et al. study(2).

3-2-2 SEM analysis

SEM analysis revealed that DTs diameter reduction was occurred in 1.6 W, 2 Wand 1.6 W+V groups, while approximately optimum sealing of DTs was occurred in 2 W+V group (figure 3-18). But at 3 W groups carbonization was noticed indicating irreversible destruction of dentin surface (figure 3-15, 3-19). This could be due to laser effect on dentin surface was intensified with high power densities, because of increased absorbed energy. The low absorption of 940 nm diode laser by dentin (52) lead to

surface temperature elevation to an accepting level (67-97.9 ◦C) for 1.6, 2 W laser with and without varnish groups, as examined by thermal camera (Table 3-10), that promote protein denaturation of dentin organic compound. The denaturant protein tend to expand (104), as a consequence, lead to DTs diameter reduction or occlusion. While for 3W groups, laser absorbed energy lead to excessive surface temperature elevation (>200) which promote carbonization and destruction of the dentin surface.

These results accepted with Liu et al study who find that 2 W/CW 980-nm diode laser, which has a close wavelength to 940nm diode laser, was a suitable power parameter due to its rapid sealing of the exposed dentinal tubules and its safety to the dental tissue, while 3W/CW produced undesirable effect on dentin surface (105).

SEM analysis also revealed that the combination groups had more sealing effect compared to laser alone groups. This could be due to a cumulative sealing effect of using varnish with laser, that was attributed to the NaF varnish mechanism of action, which relies on interactions occurring between NaF in varnish and calcium ions from saliva or varnish resulting in calcium fluoride crystals (CaF2) precipitation on the dentinal tubules that could mechanically occlude the tubules opening. Beside diode laser absorption was intensify with varnish addition, due to increased mineral content, and hence more thermal effect on dentin surface. But it may provoke cracks and destruction at high power (3 W). In addition, in the combination groups it was found that, the number of occluded DTs were more than in varnish alone group. This effect seems to be due to a high increase in fluoride uptake depth in dentin after absorption of laser irradiation by varnish via its mineral contents (figure 3-20), as reported by Mei et al (106), leading to more calcium fluoride formation, more adhesion and consequently better sealing effect.

This high increase in fluoride uptake depth of varnish by laser, reflect its resistance to be brushed away by manual tooth brush after 10s brushing that was done after lasing, while it could be brushed away when used alone as investigated previously under SEM (figure 3-10, 3-11). The combination treatment appeared to be a step forward to reduce the shortcoming of either treatment alone.

These findings came in accordance with Lan et al, Tosun et al studies, which revealed that the combination treatment of sodium fluoride varnish and Nd: YAG laser, which occur in NIR region as 940nm diode laser, resulted in more occluded dentinal tubule orifices on dentin surface structure than when used alone, and the varnish could be brushed away when used alone especially from the center of orifices (107, 108)

3-2-3 Permeability test

This investigation was performed to evaluate quantitatively the permeability of human dentin discs before and after application of varnish, laser or laser with varnish, since the tubules may seem to be occluded at SEM micrographs, but not certainly being sealed.

The result revealed that there is a significant difference between control group and 1.6W+V, 2W , 3W groups ($P< 0.05$), while the high significant difference occurred between control group and 2W+V, 3W+V groups ($p< 0.01$), and no significant difference for the varnish alone group with control group (table 3-15). This may indicate that, the permeability decrease as power density increase which could be due to increased absorbed energy and hence more laser effect on dentin surface and consequently more sealed DTs, and the application of NaF white varnish had a synergistic effect in reducing dentinal permeability when used with laser. These results reflect that, in the combination groups the sealing effect on the DTs is higher than each treatment alone, and according to Hagen-Poiseuille's law, the

dominating factor in permeability is the tubular radius, which influence the dentinal fluid flow in the fourth power. A reduction in dentinal fluid flow (permeability) diminish the nerve endings excitability upon stimulus that evoked fluid movements and pain perception.

These findings accepted with Rizzante et al. study which demonstrated a better result in permeability reduction with increased 980 nm diode laser irradiation power, which is the close wavelength to 940 nm, and the combination of diode laser irradiation with a fluoride varnish ensuring a greater reduction in permeability (109)

3.3 Conclusions

1- The combined application of 940 nm diode laser with sodium fluoride white varnish with tri-calcium phosphate showed a significant improvement in their effects on the :

   a. Dentinal tubules sealing as proved with SEM micrographs

   b. Dentin permeability reduction as displayed by diminished dye penetration depth compared to each varnish, or laser treatment alone.

2- The results of this study confirmed that 940 nm diode laser irradiation at (2 W) with sodium fluoride white varnish with tri-calcium phosphate, at 809.7 W/cm^2 power density, in continuous mode, for 10 sec, and laser fiber tip diameter: 300 μm lead to a successful and safe sealing dentinal tubules, while at high power (3W) it provokes dentin surface destruction.

3.4 Suggestions for future work

1. Clinical study (in vivo) on dentin hypersensitivity treatment using 940 nm diode laser combined with sodium fluoride white varnish with tri-calcium phosphate.
2. Trial of use another desensitizing agent in a combination with 940 nm diode laser in dentinal tubules sealing.
3. Remineralizing effect of sodium fluoride white varnish with tri-calcium phosphate alone or combined with 940 nm diode laser on initial enamel lesion.

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Appendix

Table 1: Samples temperature measurements records (°C) among laser tested groups.

Power	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
0.8W	0.9	0.4	0.9	0.6	1	0.8	1.1	0.4	0.5	0.6
1.6W	1	1.6	0.7	0.8	0.6	2.8	2.9	2.9	2.1	1.4
2W	0.8	1.3	1.79	1.2	3	2.8	1.3	1.1	1.9	2.9
3W	1.9	0.5	1.2	1.1	2.4	2	1.3	1.4	1.9	1.4
0.8W+V	0.7	0.4	0.2	0.4	1	0.6	0.6	0.7	0.4	0.6
1.6W+V	2.7	2.7	0.4	1.1	1.4	0.6	0.9	1.6	2.4	0.9
2W+V	2.7	0.8	2.1	1.6	1.5	1.9	1.1	1	2.8	1.1
3W+V	1.1	1.2	0.6	1.3	2.1	1.4	2	0.5	1.7	1.9

Table 2: Samples thickness measurements (mm)

Groups	1	2	3	4	5	6	7	8	9	10
0.8W	1.77	2.08	1.8	1.92	1.7	1.86	1.68	2	1.98	1.94
1.6W	1.98	1.84	2.1	2.05	2.2	1.68	1.54	1.66	1.76	1.86
2W	2.1	1.83	1.82	1.94	1.6	1.7	1.88	1.98	1.77	1.68
3W	1.79	2.12	2.05	2	1.66	1.68	1.98	1.94	1.82	1.93
0.8W+V	1.66	1.95	2.13	2.11	1.64	1.82	1.84	1.69	1.98	1.86
1.6W+V	1.72	1.67	2.1	1.97	1.94	2	1.99	1.92	1.83	1.98
2W+V	1.66	2.18	1.72	1.83	1.88	1.76	1.96	2.14	1.62	1.93
3W+V	1.98	1.95	2.04	1.91	1.6	1.88	1.63	2.09	1.76	1.73

Table 3: Data of dye penetration ratios among all tested groups

Groups	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Control	38.8	32.006	22.7	16.3	32.8	50.8	32	37.2	9.53	20.1
Varnish	42.2	8.14	2.83	21.5	34.5	25.11	32.8	29.8	2.93	5.67
0.8W	24.4	42.8	21.5	15.5	10.4	40.7	7.35	30.7	2.02	30.5
0.8W+V	15.6	33.1	10.97	16.4	9.17	2.68	29.4	1.85	14	2.38
1.6W	1.22	20.42	8.73	11.6	8.07	20.4	6.49	6.52	18.3	26
1.6W+V	25.4	9.011	7.17	16.2	5.09	20.2	3.66	5.83	2.21	3.64
2W	13	8.81	4.21	20.8	23.1	0.6	5.41	6.79	10.9	3.6
2W+V	3.83	13.5	2.82	19.3	9.49	4.1	0.42	9.63	10	3.85
3W	17.5	5.6	14.3	9.14	2.44	15.3	0.46	20.6	6.7	3.3
3W+V	11.3	6.98	2.65	9.9	9.07	2.05	0.39	5.18	11.6	17.2

الخلاصة

المقدمة: واحدة من أكثر السمات الشاذة للمركب اللبي العاجي هوفرط الحساسية. وقد وجد أن الأسنان شديدة الحساسية لديها عدد أكبر وقطر أوسع من أنابيب العاج من تلك المتواجدة في الأسنان غير الحساسة. وقد اقترح الليزر لعلاج فرط الحساسية، و فيما يتعلق بتأثير الليزر على أنابيب العاج ، يختلف تبعا للاطوال الموجية، وضوابط التشعيع، وتقنيات التطبيق.

الأهداف: هدفت هذه الدراسة إلى المقارنة بين تأثيرات ليزر الدايود ٩٤٠ نانومتر بكثافات طاقة مختلفة، مع أو بدون ٥٪ طلاء فلوريد الصوديوم الأبيض مع ثلاثي كالسيوم الفوسفات، على غلق نهايات الأنابيب العاجية المكشوفة ، وعلى نفاذية العاج ، واقتراح إعدادات آمنة من ليزر الدايود٩٤٠ نانومتر لغلق الأنابيب العاجية بالنسبة الى أنسجة الأسنان.

المواد وطرق العمل: استخدمت للدراسة مائتان وستة عشر من الأسنان الضواحك العليا البشرية بعد ازالة طبقة الملاط. تم تقسيم العينات إلى ١٠ مجموعات: مجموعة السيطرة ومجموعة الطلاء فقط, ومجموعات الليزر بدون ومع الطلاء عند ٠,٨ و ١,٦ و ٢ و ٣ واط (كثافة الطاقة: ٣٢٣,٨، ٦٤٧، ٨٠٩,٧، ١٢١٤,٥٧ واط / سم ٢ على التوالي)، مدة التعرض: ١٠ ثوان ، قطر الألياف البصرية: ٣٠٠ مايكرومتر؛ الوضع المستمر و بدون تماس. استخدمت ستة وتسعون من الأسنان لتقييم ارتفاع درجة الحرارة أثناء تشعيع الليزر,منها عشرة اسنان لكل مجموعة لقياس درجة حرارة اللب,ومنها اثنتين من العينات لكل مجموعة لقياس حرارة السطح الخارجي باستخدام الكاميرا الحرارية .تم استخدام مائة من الأسنان، عشرة لكل مجموعة، لدراسة نفاذية العاج عن طريق قياس اختراق صبغة المثيلين خلال العاج .وقد فحصت عشرين عينة، اثنان لكل مجموعة، تحت المجهر الإلكتروني الماسح لتحليل التغيرات السطحية تحت قوة تكبير X٢٠٠٠ ، X ٥٠٠٠.

النتائج: أظهرت قياسات ارتفاع درجة حرارة اللب أن أقصى زيادة في درجة الحرارة كانت ٣ درجة مئوية، مع متوسط سمك العاج ١,٨٧١ ملم. وبالنسبة لارتفاع درجة حرارة السطح الخارجي، كشفت النتائج أن ارتفاع درجة الحرارة، لكل من مجموعات الليزر وحده والليزرمع الطلاء، كان ما بين (٦٧-٩٧,٩ درجة مئوية) عند ١,٦ و ٢ واط، وأنه يتجاوز ٢٠٠ درجة مئوية عند ٣ واط مما يشير إلى حدوث تفحم السطح. وفيما يخص تقييم نفاذية العاج قد لوحظ وجود فرق معنوي بين مجموعة السيطرة ومجموعات ١,٦ واط مع الطلاء ,٢ واط ,3 واط .بينما اظهرت النتائج وجود فرق معنوي كبير بين مجموعة السيطرة ومجموعتي ٢واط مع الطلاء و٣ واط مع الطلاء كلاهما اظهر نفس الفرق المعنوي وليس هناك فرق معنوي لمجموعة الطلاء وحدها .وكشف تحليل المجهر الالكتروني الماسح أن التضيق في قطرالانابيب حدث في مجموعات ١,٦ واط, ٢ واط , و١,٦ واط + V، في

حين أن ما يقرب من الاغلاق المحكم من الانابيب حدث في مجموعة ٢واط مع الطلاء,ولكن قد لوحظ تفحم سطح العاج عند ٣ واط.

الاستنتاجات: استنتاجا للدراسة المعنية، تم التوصل الى ان التطبيق المشترك لليزر الدايود ٩٤٠ نانومترباستخدام ٢ واط , كثافة الطاقة ٨٠٩,٧ واط / سم ^{2}, مع طلاء الصوديوم فلورايد مع ثلاثي كالسيوم الفوسفات أظهرت تحسنا كبيرا في تأثيرها على ختم الأنابيب العاجية كما اثبت من قبل صور المجهر الالكتروني الماسح وعلى الحد من نفاذية العاج كما تم توضيحه من قبل انخفاض عمق اختراق الصبغة ، مقارنة بكل معامل وحده .

وزارة التعليم العالي والبحث العلمي
جامعة بغداد
معهد الليزر للدراسات العليا

تأثيرات ليزر الدايود ٩٤٠ نانومتر مع او بدون الطلاء على الانابيب العاجية و نفاذية العاج
(دراسة مختبرية)

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بكالوريوس طب وجراحة الفم والاسنان -٢٠١٠

باشراف

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