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DEPARTMENT of PERIODONTOLOGY

Effects of Er:YAG and CO_2 Lasers With and Without Sodium Fluoride Gel on Dentinal Tubules: An *In Vivo* Scanning Electron Microscope Analysis

Doctorate Thesis

B.D.S
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II) CONTENTS

III) ABBREVIATIONS

Å:	Angstrom
CO_2:	Carbon Dioxide
Er:YAG:	Erbium: Yttrium-Aluminum-Garnet
et al.:	el alibi
F:	Fluoride
hr:	hour
Hz:	Hertz
J:	Joule
M	molar
min:	minute
mJ:	millijoule
mm:	millimeter
MPa:	Mega Pascal
msec:	millisecond
Na:	Sodium
Nd:YAG:	Neodymium: Yttrium-Aluminum-Garnet
nm:	nanometer
sec:	second
SEM:	Scanning Electron Microscopy
W:	Watt
µg:	microgram
µm:	micron
° C:	Centigrade Degree

1. Summary

Dentinal hypersensitivity is one of the most disturbing chronic complaints in dentistry; clinicians have been trying to manage this problem for the last century. However, only little progress has been achieved due to the inability to determine the nature of this problem. Dentinal hypersensitivity has been shown to be directly related to the number and size of the exposed dentinal tubules which allow the passage of the stimulus to the pulp. With the recent development of laser technology and their applications in dentistry, the main goal of this study is to evaluate the potential of Er:YAG and CO_2 laser with or without fluoride to occlude the open dentinal tubules.

Thirty-six teeth indicated for extraction due to severe periodontitis were used in this study. The roots of all teeth were prepared by forceful scaling and 1% citric acid application for 1 min to expose the dentinal tubules. Then teeth were divided into six groups where group I received no further treatment and group II was treated with 2% NaF gel only. Group III received Er:YAG laser (50 mJ, 3 Hz) while group IV received CO_2 laser (0.5 W, continuous wave) treatments for the prepared root surface. Groups V and VI received similar laser treatments as groups III and IV, respectively, following the application of 2% NaF gel to the root surface. Then teeth were extracted atraumatically and prepared for scanning electron microscope evaluation of the treated surfaces.

Dentinal tubules were successfully exposed in the control group I; the diameters of the tubules had a mean of 1.96 ±0.60 and the number of open dentinal tubules was 1.19 per 100 μm^2. Group II exhibited narrowness of the diameters of the dentinal tubules (mean 1.12 ±0.30) and the number of open dentinal tubules was 0.94 per 100 μm^2. In group III, the surface of the root seemed smooth with the rare presence of open tubules. In group IV, a high melting appearance has caused a change of surface appearance giving a whirl-like structure around each supposed dentinal tubular entry. In group V, closure of dentinal tubular orifices was achieved with the formation of an amorphous surface. In group VI, an irregular but smooth melted surface covering dentinal surface occurred with

the absence of open dentinal tubules. For diameters of dentinal tubules, the results from combination groups revealed statistical significant difference compared to the control group (Group I vs group V and group I vs VI). For numbers of open dentinal tubules, group I showed statistical significant difference compared to the groups III, IV, V, and VI, also group II showed statistical significance compared to groups III, IV, V, and VI.

All laser treatments gave the photomicrographic image that suggests the reduction of hypersensitivity in the examined teeth. The additional effect of fluoride seems to be beneficial as fluoride might have engaged in the new structure formed after melting of the dentinal surface. However, these effects' reflection on hypersensitivity remain theoretical and need to be supported by further comprehensive clinical studies.

2. Introduction

2.1. Root Surface of the Tooth: Structure and Physics

Cementum is a hard calcified dental tissue covering the anatomic root of the tooth. It contains no blood or lymph vessels and has no innervation. Its color is light yellow which is slightly lighter than that of dentin. It is formed by cells known as cementoblasts which develop from undifferentiated mesenchymal cells in the connective tissue of the dental follicle (32). It is slightly softer than dentin and consists of about 45 - 50% inorganic material (hydroxyapatite) and 50 - 55% organic material and water by weight. Cementum has the highest fluoride content of all mineralized tissue.

Cementum deposition is a continuous process that proceeds at varying rates throughout life. Cementum formation is most rapid in the apical regions, where it compensates for tooth eruption and attrition. The thickness of cementum on the coronal half of the root varies from 16 - 60 μm. It is thicker in distal surfaces than in mesial surfaces because of functional stimulation from mesial drift (20). Between the ages of 11 and 70, the average thickness of the cementum increases three fold, with the greatest increase in the apical region (32).

Cementum covers the root part of dentine while enamel covers the dentine in the anatomical crown area. The cemento-dentinal junction is a smooth area in the permanent tooth which forms a firm attachment of cementum to the dentin. The cementum joins the enamel to form the cemento-enamel junction, which is referred to as the cervical line. In about 10% of teeth, enamel and cementum do not meet, and this can result in a sensitive area.

Dentin is the hard tissue portion of the pulp-dentin complex and forms the bulk of the tooth. It is formed by odontoblasts which are considered a part of both dentin and pulp tissues. Their cell bodies are in the pulp cavity but their long, slender cytoplasmic cell

processes extend into the tubules in the mineralized dentin. These odontoblastic cell processes let the dentin to be considered as a living tissue with the capability to react to physiologic and pathologic stimuli. Such stimuli can result in changes throughout the life of the tooth, such as secondary dentin, reparative dentin, and sclerotic dentin.

Chemically, mature dentin is approximately 70% inorganic material, 20% organic material, and 10% water by weight, and 45%, 33%, and 22% by volume, respectively. The inorganic component consists mainly of hydroxyapatite, and the organic phase is type I collagen with inclusions of glycoproteins, proteoglycans, and phosphoproteins. Dentin is less mineralized than enamel but more mineralized than cementum or bone. The mineral content of dentin increases with age. Dentinal crystallites are smaller than enamel crystallites, having a length of 200 to 1000 Å and a width of about 30 Å, similar to the sizes seen in cementum and bone (14).

The hardness of dentin averages one fifth that of enamel, and its hardness near the dentinoenamel junction is about three times greater than near the pulp. While dentin is a hard, mineralized tissue, it is somewhat flexible, with a modulus of elasticity of 242 x 10^6 MPa. This flexibility helps support the more brittle, nonresilient enamel. The tensile strength of dentin is approximately 40 MPa, which is less than cortical bone and approximately one half that of enamel. The compressive strength of dentin is much higher reaching up to 266 MPa (14). Dentin has yellowish color. Light can readily pass through thin, highly mineralized enamel and be reflected by the underlying dentin resulting in the yellowish appearance of the crown. Teeth with pulp disease often show discoloration of the dentin, which causes a darkening of the tooth.

The dentinal tubules are small canals that extend across the entire width of the dentin from the dentinoenamel or dentinocemental junction to the pulp. Each tubule contains an odontoblast's cytoplasmic cell process known as Tomes fiber. The ends of the tubules are perpendicular to the dentinocemental and dentinoenamel junctions. Each dentinal tubule is lined with a layer of peritubular dentin, which is much more mineralized than the surrounding intertubular dentin. The fluid in these tubules comes from the intercellular

fluid of the pulpal connective tissue; it is clear as water and has a composition similar to that of synovial or cerebrospinal fluid. Since the odontoblasts form dentin, while progressing inward toward the pulp, the tubules are forced closer together. The number of tubules increases from 15,000 - 20,000/mm^2 at the surface to 45,000 - 65,000/mm^2 at the pulp (29). The lumen of the tubules also varies from the dentinal surface to the pulp. In coronal dentin, the average diameter of tubules at the dentinoenamel junction is 0.5 - 0.9 μm, but this increases to 2 - 3 μm near the pulp.

Smear layer is another structure found on the root surface. It contains microcrystalline debris produced by scaling, abrasion, attrition, and caries. It extends slightly into the exposed dentinal tubules, covers the dentinal surface, and is usually several microns in thickness. This debris is mixed with saliva, water, and dentinal fluid. Such a closure of the dentin surface reduces both sensitivity and permeability. In a way this is a defense mechanism, even though it is not related to dentinoblastic function. Organic solid material and microorganisms may also contribute to the plugging of the outer tubule apertures. According to Brannstrom (15), after several weeks and with the assistance of dentinal fluid and substances from the saliva, thin mineralized areas may be evident on the exposed dentin. This phenomenon is comparable to the development of calculus and the remineralization of enamel caries; it may not occur when there is too much acid food, defects in the composition of saliva, and overzealous brushing (15).

2.2. Dentinal Hypersensitivity

Dentinal hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli typically thermal (hot or cold), evaporative, tactile (tooth brushing and digital probing), osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology (40). Occasionally, it may persist for a variable time as a dull or vague sensation in the affected tooth after removal of the stimulus. Patients often direct the operator to the sensitive areas. These may be located by gentle exploration with a probe or cold air.

Hypersensitive dentin occurs due to the transmission of pain-producing stimuli as a result of opened apertures of dentin tubules. Vasodilatation or inflammation does not need to be present in the underlying pulp tissue. Hypersensitivity may be associated with exposed dentinal tubules as a result of abrasion, erosion, or following root planing or it may be due to lowered pain threshold of peripheral receptors in the pulp as a result of underlying prolonged vasodilatation or local inflammation. Different theories have been suggested to explain the mechanism of transmission of this stimulus and will be explained in the next section.

It is estimated that pain due to tooth hypersensitivity is a persistent problem that affects as many as one of every seven dental patients, and the incidence tends to peak around the third decade of life and is equally divided between men and women (33). It is prevalent amongst a large portion of individuals 30 to 40 years old, and by causing oral discomfort generated by pain, it leads to nutritional deficiency due to dietary restrictions in some individuals (53). Calculations showed that 40 million Americans may have some degree of dentin hypersensitivity at some point in their life (94), while others reported that the prevalence of dentinal hypersensitivity in other countries may approach 50% of the population (31, 78). The cervical area of teeth is the most common site of hypersensitivity. With the population living longer and retaining their natural teeth, hypersensitivity is expected to become a growing concern because of the increased number of teeth with attrition, abrasion, and erosion.

Dentin hypersensitivity is usually a chronic condition with acute episodes: dentin that is freshly cut or has recently been curetted will respond to the same cold, heat, osmotic, and acid stimuli in an acute manner. Following cavity preparations, or curettage, the dentin may heal by the action of calcification mechanisms that block the tubules, but if healing does not occur, the tooth may respond chronically to stimuli that are usually considered not noxious (19).

2.3. Theories of Pulpa-dentinal Pain Excitation

For the mechanism of transmission of the stimulus through the dentin to initiate a nerve impulse, three theories or hypotheses have been proposed: (1) dentin innervation, (2) dentinoblastic deformation or injury (transduction), and (3) hydrodynamic mechanism. However, the mostly accepted explanation is the hydrodynamic theory.

2.3.1. Dentin innervation

The theory of dentin innervation states that there are nerve fibers within the dentinal tubules that, when injured, initiate the nerve impulse. However, the extent of observable nerve fiber penetration has been limited to the predentin and inner dentinal zones known as plexus of Raschkov. Penetration of the nerve fibers all the way through the dentin has not been observed with any certainty or consistency. The question is whether the intratubular nerves are involved in dentin sensitivity. No evidence has been found for nerves in the outer dentin, which is reputedly the most sensitive. Development studies have shown that the plexus of Raschkow and the intratubular nerves do not establish themselves until some time after the tooth has erupted, yet newly erupted teeth are sensitive.

Experimental evidence, as well, suggests that nerve fibers are not present in peripheral tubules (6, 13, 81). Such evidence includes:

1. Pain-producing pharmacological agents that cause pain when applied to skin such as KCl, bradykinin, acetylcholine, and histamine do not produce pain when applied to exposed peripheral dentin.
2. Hypertonic NaCl solution, capable of depolarizing nerve membranes in the deeper layers of dentin or the exposed pulp, will not initiate a nerve impulse when applied to peripheral dentin.
3. Application of local anesthetics or silver nitrate (a protein precipitant) to exposed dentin does not eliminate dentin sensitivity.

2.3.2. Dentinoblastic deformation or injury (transduction)

The second mechanism to explain dentin sensitivity considers the dentinoblast to be a receptor cell. This concept has been first abandoned and then reconsidered. The argument rises from the fact that dentinoblasts are of neural crest origin and can transduce an impulse. The missing part was the synaptic relationship between the odontoblasts and the pulp nerves. However, it was observed that dentinoblasts can be injured by any stimulant applied to the dentin: thermal, mechanical, chemical, or osmotic. The dentinoblast and its process may function as a transducer mechanism when the membrane stimulation is transformed into a chemical or electrical message. Dentinoblasts would not act as receptors of stimuli; when deformed or injured, they may produce stimuli that are received by the nerve endings near the dentinoblast. The stimulus produced by the dentinoblasts may be due to chemicals released by the injured cells, changes in their electrical surface potential, or movement associated with their deformation.

1. **Chemoactivation:** The injured dentinoblast may release polypeptides that cause the naked nerve fibers to fire off an impulse. The produced chemicals apparently combine with unmyelinated pain fibers in the involved area, altering their permeability and starting an impulse (action potentials). The presence of "coated pits" on dentinoblastic processes suggests a stimulus transmission process through chemoactivation.

2. **Electroactivation:** An injury to the dentinoblastic process changes the electric surface charges of the plasma membrane at the point of injury. These changes travel along the plasma membrane and stimulate pain receptors in contact with any portion of the dentinoblast. Because of the highly calcified peritubular matrix, the action potentials are confined to spread within the tubules.

3. **Mechanoactivation:** The mere movement of the dentinoblasts may move the Aδ terminal fibers, which have a low threshold of excitability. This movement increases the nerve cell's permeability to sodium ions. The resultant rapid inward movement of the sodium depolarizes the fiber membrane, and an action potential is initiated. Hydrodynamics may not be responsible for the pain associated with light brushing of an explorer tip across exposed dentin because of insufficient tubular fluid displacement and flow. Pain is due to direct stimulation of the dentinoblastic processes, which serve as transducers, transferring the stimulus to the nerve fibers. So when dentin is stimulated, a morphologic change occurs in the dentinoblastic process (84). This then transmits the stimulation to the nerve fibers, resulting in pain.

2.3.3. Hydrodynamic mechanism

Currently, the most accepted explanation of dentinal sensitivity is the hydrodynamic mechanism (1, 13, 14, 23). It was first proposed by Gysi (37) in 1900. Later on in the 1960s, Brannstrom (14) supported this hypothesis with experimental and morphological data. The hydrodynamic theory suggests that sensitivity occurs due to movement of fluids through dentinal tubules. The naked nerve endings in the pulp are exquisitely sensitive to sudden pressure changes, fluid movement, or mechanical deformation. It makes no difference whether the source of movement or pressure is from inside the pulp or is transmitted through the dentinal tubules.

Brannstrom (14) has proposed that dentinal pain is due to a hydrodynamic mechanism. The dentin contains more than 300,000 capillary tubes per square millimeter and constitutes about 10% of the dentin's volume. This percentage is higher near the pulp than peripherally. The fluid in these tubules comes from the intercellular fluid of the pulpal connective tissue. The tubular fluid obeys the same laws of physics as do liquids in glass capillaries. Any displacement, no matter how slight, causes a flow of intratubular fluid. A rapid displacement in thousands of tubules at the same time produces a corresponding movement in the tubules as well as a significant movement in the contiguous pulpal

tissue. This movement, either pulp-ward or outward, exerts a direct mechanical deformation on the low-threshold A-δ free nerve fibers within the tubules or in the subjacent pulp tissue and an action potential of pain impulse is initiated.

Any stimulus that extracts tubular fluid from its outer surface causes an outward flow. The lost fluid is immediately replaced by pulpal tissue fluid responding to the capillary force within the dentinal tubules. Heat caused by a bur during cavity preparation dehydrates the dentin (evaporation). This stress may also mechanically press fluid out of the tubules. The pressures of chiseling or scraping the surface may have the same effect. In addition to the outward flow produced by these agents, dentinoblasts and nerve terminals may also be stretched or aspirated into the tubules, evoking a painful response.

Candy bars and other sweets frequently trigger pain from a tooth with a defective restoration. Sugar and hypertonic solutions create an osmotic gradient, causing fluid movement from the deeper tubular areas of lesser concentration. The initial surge of dentinal tubular fluid results in firing of the low-threshold A-δ fibers and a resultant sharp pain. If a concomitant inflammation is present in the subjacent pulpal tissue a dull persistent ache may follow as a consequence of activation of the higher-threshold C fibers.

In a healthy tooth, the pain associated with thermal stimulation may be due to the movement of fluid within the tubule, since fluids have a coefficient of expansion about 10 times greater than that of the tubule wall. Cold causes a contraction of the fluid and its outward flow, whether the tubule is open or closed at its outer surface. Heat, on the other hand, causes expansion of the fluid and movement toward the pulp if the tubule is closed at the outer surface (i.e., covered by enamel or cementum). A painful response to cold is faster than to heat because there is rapid outward movement of the tubule contents, whereas with heat a larger volume of the dentin must be affected before a sufficient pronounced dislocation of the tubule contents is produced.

2.4. Management and Treatment of Dentinal Hypersensitivity

Exposure of the dentin may result from one of two processes, either removal of the enamel covering the crown of the tooth, or denudation of the root surface by loss of cementum and overlying periodontal tissues. Removal of the enamel may result from attrition relating to occlusal abnormalities, toothbrush abrasion, dietary erosion, habits, or a combination of these factors. Denudation of the root surface is multifactorial; periodontal diseases, resective periodontal surgery, incorrect tooth brushing and chronic trauma from habits are of particular importance. Orchardson and Collins (85) reported that gingival recession was significant in 68% of hypersensitive teeth, where only 25% had evidence of abrasion, attrition or erosion.

Proper differential diagnosis must be considered before concluding that a dental pain is dentinal hypersensitivity (22, 40). For most patients, dentinal hypersensitivity which typically follows instantaneously upon application of the offending stimulus is sharp, transient short-lived and usually resolves immediately after withdrawal of the stimulus (23). Occasionally the pain may be more severe and persist for a variable time after removal of the stimulus. Although sometimes pulpal inflammation complicates the symptomatology, tooth hypersensitivity differs from pain arising in the pulp due to inflammation. Patients can readily locate the source of discomfort or pain when a stimulus is applied to a hypersensitive tooth. On the other hand, pulpal pain is lasting, intermittent and throbbing. Stimuli such as heat, cold, osmotic change and acid can start an episode of pulpal pain that may last few minutes to several hours and is usually difficult to locate. Chewing can also be a stimulus for pulpal pain.

Several other conditions may elicit the same clinical symptoms as dentinal hypersensitivity. These include: cracked tooth syndrome, fractured restorations, chipped teeth, dental caries, postrestorative sensitivity, palatal-gingival groove, hypoplastic enamel, a congenitally open cementum-enamel junction, improperly insulated metallic restorations and teeth in acute hyperfunction. A careful diagnosis, therefore, is required to

rule out alternative causes of pain. This should include a history and a thorough clinical and radiographic examination of the tooth in question, as well as the adjacent teeth.

It is noteworthy that although many individuals are seen to have exposed dentin, not all experience symptoms. There is no clear-cut explanation for this apparent anomaly, nevertheless certain factors may be responsible, in those individuals where no symptoms arise from dentin exposure, occlusion of tubules may have resulted from the formation of dead tracts with the laying down of irregular secondary dentin, or the development of sclerosed dentin (46). Similarly, blockage of the tubules at the dentin surface by other means may occur and include dentifrice ingredients and oral debris (38).

Besides directly causing patient discomfort, dentinal hypersensitivity may indirectly deter a person from establishing or maintaining adequate oral hygiene procedures, further complicating oral health (38, 98). The failure to practice satisfactory plaque control has well-established consequences in respect of gingival health (62). On the other hand, excessive brushing force is also a risk factor for evoking and worsening dentinal hypersensitivity; overzealous brushing may cause the abrasion of the tooth and/or gingival recession, followed by the cervical dentin or root surface being exposed to the oral environment. However, if plaque control is performed adequately, dentinal hypersensitivity gradually disappears with time (99). Thus, hypersensitive patients should be encouraged to continue brushing their teeth in a proper way which prevent accumulation of plaque, and at the same time not cause further loss of dentin (22). Excessive force, hard toothbrushes, highly abrasive toothpastes should be avoided. The use of none or low abrasive dentifrices or brushing with water help in closure of dentinal tubules; while brushing with a dentifrice containing calcium hydrogen phosphate as an abrasive system leads to opening of the dentinal tubules (52). Toothbrushing should be avoided after consuming acidic foods and drinks since tooth brushing, in combination with acid decalcification of superficial dentin, is capable of accelerating the loss of tooth structure (21) and opening dentinal tubules (2). For this reason, the currently available evidence strongly contradicts brushing immediately after meals (2, 4, 21).

Dietary acids have a clear role in the initiation of dentinal hypersensitivity. It has been demonstrated that acidic substances are able to remove the smear layer and open dentinal tubules (92, 103). Therefore, dietary counseling should be considered for the management of the dentinal hypersensitivity. A written diet history obtained from patients with hypersensitivity may be useful in order to identify etiological agents and form a basis from which to provide advice (22). Suggestions to drink something neutral or alkaline after consuming acids such as water or milk, to avoid sipping, to use a straw, to chill the drink which can reduce its erosive potential and to avoid acids as a snack just before bed time should be explained to the patient (83).

Dentinal hypersensitivity can provoke emotional distress and chronic discomfort when constantly present over a long time (11, 82). Pain arising from exposed dentin is very subjective and may be further modified by psychological factors. This makes the assessment of the extent of the problem sometimes difficult. Pain threshold of the patient depends on his/her anxiety. Thus, good dentist-patient communication is vital in the management of dentinal hypersensitivity; explaining to the patient the possible causes of hypersensitivity increases the likelihood of patient compliance, decreases the patient anxiety and improves the probable outcome of treatment (83).

Professional interest in the causes and treatment of dentinal hypersensitivity has been evident in the literature for the past 150 years (93). In 1935, Grossman (36) suggested a number of requirements for treatment of dentinal hypersensitivity. According to Grossman, the therapy should be non-irritant to the pulp, relatively painless on application, easily carried out, rapid in action, effective for a long period, without staining effects, and consistently effective. These criteria still hold true today, but most of the present therapies have failed to satisfy one or more of these requirements. Generally, two principal therapeutic aims are considered in the treatment: obturating open dentinal tubules or inhibiting pulpal nerve responses (93). A very wide variety of agents have been used to achieve the aforementioned therapeutic goals. These agents can be categorized according to delivery method to home-use and professional (in-office) products. Home use agents are present in over-the-counter products prescribed in the

form of mouthwashes and toothpastes (44). On the other hand, professional products have been classified according to their chemical and physical properties as shown in Table 1.

Table 1. Various agents used for in-office treatment of dentinal hypersensitivity (94).

Chemical agents	**Physical agents**
Corticosteroids	Composites
Silver nitrate	Resins
Strontium chloride	Varnishes
Formaldehyde	Sealants
Calcium hydroxide	Glass-ionomer cements
Potassium nitrate	Soft tissue grafts
Fluorides	Lasers
Sodium citrate	
Iontophoresis with 2% Sodium fluoride	
Potassium oxalate	

Physical agents form a barrier which would physically isolate the dentinal surface from the oral environment and produce a macroscopic coat on the dentinal surface. But the mechanisms by which chemical agents decrease sensitivity are either unknown or unproven. Many possibilities have been suggested; such as occlusion of the orifices of dentinal tubules, protein precipitation, increasing peritubular dentin mineralization, or inducing the formation of sclerotic dentin (35).

Fluoride compounds (58, 98), calcium hydroxide (26), formulations containing potassium ions (86, 91), anti-inflammatory agents (73), silver nitrate (5), and cyanoacrylate (45) are the main chemical agents evaluated in the dental literature. A brief review about these agents will be discussed in the following paragraphs.

Sodium fluoride (NaF) was first proposed as a desensitizing agent by Lukomsky (63) in 1941. Studies (41, 63, 79) have shown that topical application of NaF exerts a beneficial, desensitizing effect on exposed dentin and cementum. Its mechanism is mainly related to

the reaction that occurs between NaF and calcium ions of dentinal fluid. This reaction leads to formation of calcium fluoride crystals, which are deposited on the dentinal tubules openings. The crystal size is small (about 0.05 micrometers), so a single application of NaF would not be effective in narrowing the diameter of dentinal tubules and multiple applications should be required. However, the effect of fluorides is transient. Therefore, attempts have been made to improve its effectiveness; one way is to incorporate it in a varnish so that it remains adherent to the tooth surface for a longer period of time (8). Kumar and Mehta (51) reported an immediate reduction of hypersensitivity in 33% of the examined teeth after the application of 5% NaF varnish.

Another way to increase the effectiveness of topical fluoride application is iontophoresis. Manning (64) described an iontophoretic device which would work electrophoretically to improve the effect of fluorides on dentin. Using 2% NaF with iontophoresis, Carlo (17) reported significant relief from sensitivity in 90% of cases in a noncontrolled study. Murthy et al. (79) compared topical 33.3% NaF to iontophoresis with 1% NaF in a controlled study. They found iontophoresisto be "good to moderate" in desensitizing the dentin immediately, while 33.3% NaF alone took 2 to 3 applications to reduce dentinal sensitivity in 85% of cases. Iontophoresis produces no favorable effects by itself. Singal et al. (98) reported a significant reduction in hypersensitivity when treated with 2% NaF iontophoresis which lasted for 3 months, although recurrence was observed in some patients. These data suggest that iontophoresis with NaF is promising for treatment of hypersensitivity. However, it should be kept in mind that the published results are for short-term.

Calcium hydroxide, which is widely used as a cavity dressing, is another chemical agent used for the management of hypersensitivity. By itself, calcium hydroxide has little or no direct effect on dentin sensory nerve activity (104), but the agent can increase peritubular dentin mineralization and with this ability the material maintains long term desensitizing effects. Mjor (72) studied the effect of calcium hydroxide application to cavity preparations in intact human premolars for 4 months. He found increased dentin mineralization in comparison to zinc oxide-eugenol, and that the calcium hydroxide

application made the dentin less permeable to dye penetration. In a 3-month clinical study, Green et al. (34) found calcium hydroxide applications consistently effective in relieving cervical hypersensitivity. Levin et al. (59) stated calcium hydroxide as an effective agent because 6 month evaluations revealed that hypersensitivity remained reduced in 40% of the 118 examined teeth.

Potassium salts have been also suggested as excellent dentinal desensitizing agents (80, 100). Hodash (39) called potassium nitrate a "superior desensitizer" after he found it was highly effective in concentrations of 1-15%. In a clinical controlled study, Tarbet et al. (100) found that 5% potassium nitrate paste was able to desensitize the teeth of 92% of the patients effectively at 1 week and up to 4 weeks which was significant compared to the control. Pillon et al. (91) studied the effect of single application of 3% potassium oxalate gel compared to a placebo gel immediately after subgingival scaling and root planning for the management of hypersensitive teeth. On day 14, reduction for the test and control groups was 64.6% and 20.5%, and on day 21 it was 81% and 34.7%, respectively. They stated that 3% potassium oxalate gel seems to be a successful agent for the treatment of hypersensitivity. Pereira and Chava (90) compared a 3% potassium nitrate/0.2% NaF mouthwash with a 0.2% NaF control mouthwash in a 6-week double-blind study. Over the 6 week study period, although general decrease in dentinal hypersensitivity levels was demonstrated in both groups. This decrease reached the significance level only in the combined group. They concluded that a 3% potassium nitrate/0.2% NaF mouthwash appears to have therapeutic potential to alleviate dentinal hypersensitivity. The exact mechanism of which potassium salts may be acting is unknown. But the potassium content may play a role directly or indirectly on the dentin neurosensory unit (16, 49). The main disadvantage of such products is the short term effect and the need to repeat the treatment over and over to prevent the recurrence of hypersensitivity.

Glucocorticoids are another choice of chemical agents that can be used for treatment of dentinal hypersensitivity. Some investigators felt that its application to cavity preparations and cervically exposed dentin would decrease dentinal sensitivity because of

its anti-inflammatory effect. In a clinical and histological study, Mosteller (77) found that the steroid was successful in reducing thermal sensitivity, but histologically it did not reduce pulp inflammation. Mjor (72) reported that steroid application to dentin was able to increase peritubular dentin mineralization. Thus, the tubule lumen would be decreased, resulting in less dentin tubule fluid movement, reducing the dentinal sensitivity. However, the mechanism of this steroid-induced mineralization is unknown and remains to be studied.

Collectively, the literature shows that clinical trials have supported divergent standpoints and found different results. It seems that the application of many agents was successful in reduction or elimination of the symptoms of dentinal hypersensitivity (8, 17, 39, 51, 72, 77, 79, 100). However, these observations shouldn't be misleading. A strong placebo effect is commonly described in such trials (109, 110). This makes analysis of the results hard and unreliable due to the overcome of the placebo effect on the true therapeutic effects of the studied agents. West et al. (108) showed that a placebo caused relief of symptoms in 40% of dentinal hypersensitivity cases due to the placebo effect in clinical trials. Other studies have confirmed these observations using water treatment as a placebo (109, 110). Another important consideration is that dentinal hypersensitivity, as any other painful condition, can improve apparently irrespective of treatment; a phenomenon described as regression of the mode. In addition, it appears that in a clinical setting the suggestion to a patient that a prescribed product is an effective treatment brings about considerable improvement, irrespective of the therapeutic potential of the formulation.

Furthermore, pain response is another factor which could affect the outcome. Pain response is subjective and may vary due to factors such as the patient's psychology and ability to express a given response. Pain may vary at different times of the day, also it may vary temporally over several months; clinical impression suggests that dentinal hypersensitivity has a somewhat cyclic pattern which may reflect changes in balance between the effects of etiological versus protective factors.

Evaluation methods can also affect the results. Tarbet et al. (101) showed that the electric stimulus was more efficient in evaluating the effect of desensitizing agent than cold air method. Clearly, in a clinical study depending on the patients evaluation of symptoms, only truly pragmatic studies could be expected to largely overcome placebo effects. In such studies, this would certainly require that the nature of the investigation be hidden from the participants. This would not only be difficult or impossible to achieve, but with today's guidelines for good clinical practice, it has significant ethical implications (110).

2.5. Laser Treatment of Dentinal Hypersensitivity

Laser is a device which transforms light of single wavelength into a chromatic radiation which could be in the visible, infrared, or ultraviolet regions with all the waves moving parallely in the same direction. Laser light is coherent; this makes it capable of delivering a high amount of energy in a small beam. Laser was introduced in 1917 by Albert Einstein and the first laser device was developed by Maiman in 1960. The word "laser" is an acronym for Light Amplification by the Stimulated Emission of Radiation. During the last few decades lasers have been used very effectively in a wide variety of different fields of medicine and dentistry.

Nearby, many other adjunctive treatment strategies in periodontology, lasers also have part in the management of dentinal hypersensitivity. Diverse array of lasers with various settings and conditions were used in the manner to treat sensitivity. Even though laser has shown positive results in many studies, the mechanisms behind this effect were not understood and still remains to be investigated. In general, the rationale for laser-induced reduction in dentinal hypersensitivity is based on two possible mechanisms that differ greatly from each other. The first mechanism involves modification of the tubular structure of the dentin by melting and fusing of the hard tissue or smear layer and subsequent sealing or obstruction of the dentinal tubules. Whereas the second implies the direct effect of laser irradiation on the electric activity of nerve fibers within the dental pulp. The clinical and histological observations of the most used lasers for treatment of

hypersensitivity will be discussed in this section with an explanation of the possible mechanisms behind these effects.

2.5.1. Carbon Dioxide (CO_2) Laser

CO_2 was first used by Kantola (48) in 1972 to create craters in dentin. Using microradiography and electron probe analysis, he showed higher levels of calcium and phosphorus in the fused or recrystallized dentin walls of the crater compared with levels in normal dentin. The relative augmentation of the inorganic content was attributed to the burning-off of the organic component by the laser energy. One year later, in a follow-up study using radiographic diffraction analysis, Kantola (47) observed that in the laser-irradiated fused dentin, recrystallization had occurred and the dentin had changed structurally so that it closely resembled the crystalline structure of normal enamel hydroxyapatite. The conversion of dentin into a crystalline structure following CO_2 laser irradiation also has been reported by others (68, 70), but the induced effect of the carbonization of organic material along with the melting of dentin cannot be overlooked (70).

Bonin et al. (12) have suggested that using CO_2 laser at moderate energy densities, can affect dentinal hypersensitivity by mainly sealing of dentinal tubules, as well as a reduction of dentin permeability. Also Moritz et al. (75) showed that CO_2 laser causes occlusion or narrowing of dentinal tubules. There have been no reports on nerve analgesia by CO_2 laser irradiation. The sealing depth achieved by CO_2 laser irradiation at 0.3 W for 0.1 sec on dentinal tubules is usually measured to be 2 - 8 μm (50).

Clinically, Moritz et al. (75) were first to use CO_2 laser in 1996, with an output power of 0.5 W in a continuous wave mode and an irradiation time of 5 sec to treat dentin hypersensitivity. They reported that the treatment effectiveness ranged from 59.8% to 100% in reduction of dentin hypersensitivity. This encouraged other investigators to use CO_2 laser in clinical studies, and favorable results were reported (74, 76, 112). Output powers of 0.5 and 1 W delivered in continuous wave were used. Irradiation time ranged

from 0.5 - 5 sec, and irradiation was repeated 5-10 times. Treatment effectiveness reports ranged from 59.8 to 100% which lasted for 3 to 6 months (74, 76, 112). These early reports seem promising and add valuable information in decision-making regarding effective treatment alternatives for hypersensitivity.

2.5.2. Erbium: Yttrium-Aluminum-Garnet (Er:YAG) Laser

The first clinical study with Er:YAG laser came from Schwarz et al. (95). They compared Er:YAG laser (80 mJ/pulse, 3 Hz), or application of desensitizing agent containing polyurethane-isocyanate 22.5% and methylenechloride 77.5% in a split-mouth design where one side served as an untreated control in each patient. Qualitative degrees of discomfort were assessed up to 6 months after treatment. Both treatment resulted in significant improvements of discomfort immediately after treatment. Authors reported that desensitizing of hypersensitive dentin with Er:YAG laser was effective for the evaluation period of 6 months. In an *in vivo* and *in vitro* study, Watanabe et al. (107) used Er:YAG laser for the treatment of dentin hypersensitivity. A low-power laser irradiation (25 to 35 mJ per pulse) was used with resulting treatment effectiveness ranging from 16% to 61%. They concluded that low-power irradiation by Er:YAG laser is effective for dentin hypersensitivity, but some limitations related to the recurrence might appear.

The desensitizing effect of Er:YAG laser was attributed to the evaporation of water content of the tooth surface which doesn't cause harm to the mineralized tissues. This would decrease the dentinal fluid movements and, according to the hydrodynamic theory, directly result in a decrease of dentinal hypersensitivity (95). Er:YAG laser has a water absorption characteristic approximately 15 times greater than that of CO_2 and even 20,000 times greater than the Nd:YAG laser (106). This property gives Er:YAG laser the ability to remove calculus without causing harm to the tooth surface (7). Many researchers have recommended the usage of Er:YAG for scaling and root planning providing evidence that no harmful effect was observed on the tooth surface (7, 25, 96). Evaluations using scanning electron microscopy (SEM) have shown that Er:YAG had no thermal effects on the root surfaces when low to moderate power outputs were used,

however morphologic changes has been reported which were attributed to the microexplosions occurring in the mineralized tooth surface due to the expansion of the water molecules following the rapid absorption of laser.

Israel et al. (43) applied three types of lasers following scaling and root planning of root surfaces of recently extracted teeth. Er:YAG was applied with a range of energy densities of 20-120 J/cm^2. The root surfaces were evaluated using SEM that showed no evidence of melting of root mineral or charring in the Er:YAG group, also there was presence of sharply defined lines of cleavage or fracture of the mineralized surface with resulting exposure of the collagen matrix. The authors suggested that the root surface resulting from exposure to Er:YAG laser was comparable to acid etched surface. This observation was also reported by other researchers (102).

Schwarz et al. (96) attempted to compare the effects of an Er:YAG laser on periodontally involved root surfaces at different power settings *in vivo* and *in vitro* using SEM observations. Forty single rooted teeth (160 surfaces), with advanced periodontal destruction that were scheduled for extraction, were divided into two groups of 80 each which were treated *in vivo* (group A) and immediately after extraction *in vitro* (group B) using one of the following energy settings: 120, 140, 160, and 180 mJ at 10 Hz (71, 83, 94, and 106 J/cm^2/pulse). The morphological changes on the treated root surfaces were evaluated using SEM observations to assess the laser induced ultrastructural changes. All surfaces treated *in vitro* (group B) showed visible crater-like defects with notch-edged borders. The depth of the surface damages varied with the power applied and was localized into cementum at energy settings of 120-160 mJ but also reached dentin at 180 mJ. Compared to that, all *in vivo* (group A) treated surfaces showed a homogeneous and smooth root surface morphology. The surface alterations were not related to the used energy setting. The authors concluded that the clinical use of an Er:YAG laser resulted in a smooth root surface morphology, even at higher energy settings. However clefts were present on the SEM views which were claimed to be artificially caused by the vacuum during SEM observations and not by the laser radiation.

The current data of Er:YAG laser favors its ability to be used for treatment of hypersensitivity. It is easy to use with minimal side effects and causes no harm to the tooth surface when proper settings are used. The exact mechanism is not clear but evaporation of the fluid in the dentinal tubules may be main effective mechanism which inhibits dentinal hypersensitivity. This is due to the high absorption of Er:YAG laser by water molecules which transforms this energy to heat and evaporates.

2.5.3. Neodymium:Yttrium-Aluminum-Garnet (Nd:YAG) Laser

The first use of Nd:YAG laser for the treatment of dentinal hypersensitivity was reported by Matsumoto et al. (65) with very high success. Later on, this laser became popular among researchers and a variety of power outputs and settings were used (30, 56, 57, 111). Black ink can be used as a stimulus which would enhance the absorption of Nd:YAG laser leading to the improved effects on the targeted surface. Some studies reported the use of black ink for enhancing the effects of Nd:YAG laser irradiation to treat dentinal hypersensitivity (30, 111), and indeed treatment effectiveness using black ink was better compared to non-use of black ink (111).

Similar to CO_2 laser, the mechanism of Nd:YAG laser effects on dentinal hypersensitivity is thought to be the laser-induced occlusion or narrowing of dentinal tubules (56, 57, 111). Direct nerve analgesia and a suppressive effect achieved by blocking the depolarization of Aδ and C fibers also were considered as possible mechanisms accounting for the effect of Nd:YAG laser irradiation in reducing dentinal hypersensitivity (87). The sealing depth achieved by Nd:YAG laser irradiation at 30 mJ/pulse and 10 Hz on dentinal tubules is estimated to be less than 4 μm (61).

Lan et al. (54) evaluated the morphologic changes of hypersensitive dentin after Nd:YAG laser irradiation *in vivo*. Thirty patients with clinically diagnosed cervical dentin hypersensitive teeth were treated with a Nd:YAG laser and an impression of the sensitive area was taken before and after laser treatment and then examined with a SEM. The impression of the dentin surface after Nd:YAG laser treatment showed no protrusive

rods, in contrast with the presence of numerous rods before laser irradiation. Because protrusive rods are a measure of open dentinal tubules, authors interpreted these data to support the hypothesis that Nd:YAG laser can be used to seal the exposed dentinal tubules. They proposed that the mechanism of the Nd:YAG laser's effect on dentin is caused by thermal energy absorption. Because the thermal energy generated by the laser was quickly absorbed by the dentin, the hydroxyapatite crystals of dentin melted partly or completely and moved and increased in size once the activation energy was sufficient. Thus, the dentinal tubules were occluded.

Ciaramicoli et al. (18) clinically followed up a total of 145 teeth for six months, where 104 received the Nd:YAG laser treatment and 41 remained as untreated control. The results showed that there was statistically significant reduction of hypersensitivity after Nd:YAG laser compared to control group. They observed that laser therapy was still effective in the treatment of cervical dentin hypersensitivity after 6 months.

Recently, Kumar and Mehta (51) evaluated the efficacy of Nd:YAG laser irradiation alone and in combination with 5% NaF varnish in the management of dentin hypersensitivity under SEM. The study was conducted on 40 patients divided into four groups who had at least one tooth of Grade III mobility with clinically elicitable dentin hypersensitivity. Following the pretreatment assessment of hypersensitivity using the visual analog scale and cold air blast test, the selected tooth in all the groups received 1% citric acid treatment for 1 min. Group 1 patients received no further treatment; group 2, 3, and 4 patients received additional treatment with 5% NaF varnish, Nd:YAG laser for 2 min, and a combination of 5% NaF varnish and Nd:YAG laser, respectively. Two hours following treatment, hypersensitivity was again assessed, and the teeth were extracted, sectioned, and scanned using SEM. The clinical symptoms in group 1 showed a 27% increase from baseline, but groups 2, 3, and 4 showed a decrease of 33%, 44%, and 62%, respectively. The mean cold air blast score showed a 22% increase in group 1, but values decreased by 43%, 50%, and 83% in groups 2, 3, and 4, respectively. They also reported that the number of patent tubules also progressively decreased from group 1 through group 4. They concluded that the combination of Nd:YAG laser and 5% NaF varnish

seems to show an impressive efficacy in treating dentin hypersensitivity. The SEM findings seemed to relate to the clinical findings in that reduction in number/patency of tubules was associated with improvement in treatment efficacy (51).

Collectively, data shows that Nd:YAG is an effective laser for the treatment of dentinal hypersensitivity. Although one study is available in the literature, combination of Nd:YAG laser with fluoride results in a more profound clinical and morphological effects.

2.5.4. Diode Laser

The first use of diode laser for the treatment of dentinal hypersensitivity was reported by Matsumoto et al. (66). Two wavelengths of diode laser (780 and 830 nm) at an output power ranging 40 - 100 mW and continuous irradiation time ranged from 0.5 - 3 min have been used for the treatment of dentinal hypersensitivity (24, 60). Treatment effectiveness rate seemed to be dependent on the output power, and was reported to range from 70 to 100%.

It is postulated that low output power diode lasers mediate an analgesic effect related to depressed nerve transmission. According to physiological experiments using 830 nm diode laser, this effect is caused by blocking the depolarization of C-fiber afferents (105). Diode laser emissions at 904 nm has an analgesic effect on the cat tongue although the mechanism remains unclear (69).

The review of literature reveals that Er:YAG, CO_2, and Nd:YAG lasers are appropriate tools for the treatment of hypersensitivity with favorable clinical outcomes (51, 74, 95). Encouraged by such results, many researchers have tried to modify the approach for the accomplishment of enhanced clinical outcomes. However, with only available data, treatment of hypersensitivity with laser still remains to be investigated. The ultimate applicability and benefits of those lasers as an alternative for treatment of dentinal hypersensitivity must be strictly evaluated based on reliable evidence and critical review

of existing literature. Among lasers, the use of Er:YAG and CO_2 is promising due to the optical properties of these lasers which can modify dentinal surfaces without harmful effects on the pulp or surrounding tissues.

3. Aim

Dentinal hypersensitivity has been shown to be directly related to the number and size of the exposed dentinal tubules. If the number and size of the exposed dentinal tubules can be diminished, success may be achieved in the treatment of dentinal hypersensitivity. Therefore, the aim of this *in vivo* study is to assess the occluding effect of Er:YAG and CO_2 lasers as a monotherapy and in combination with topical NaF gel on human dentinal tubules by SEM.

4. Materials and Methods

4.1. Subjects and Teeth

Twenty-four patients who applied to the clinics of Periodontology at the Faculty of Dentistry, Yeditepe University, were enrolled in this study. Patients were not included if they were using any desensitizing agent within the past six months. From the 24 patients, 36 anterior teeth indicated for extraction due to severe periodontitis were employed in the study after the patients have went through non-surgical periodontal therapy. Teeth were excluded from the study if they were not vital, cracked, or had large carious lesions or restorations. After an explanation of all aspects of the study, the patients were required to sign an informed consent. The study and consent were approved by the University Institutional Review Board.

4.2. Study Groups

The study had a parallel design. Six treatment groups were used: I) no treatment, II) NaF 2%, III) Er:YAG laser, IV) CO_2 laser, V) Er:YAG laser + NaF 2%, VI) CO_2 laser + NaF 2% as shown in Table 2.

Table 2. Treatment groups and distribution of patients and teeth.

Group	Number of teeth	Treatment	Number of patients and number of teeth from each patient	Types of teeth
I	6	Control	3 x 2	4 lower central incisors 1 upper central incisor 1 upper lateral incisor
II	6	NaF 2%	2 x 2 2 x 1	3 lower central incisors 2 upper lateral incisor 1 upper canine
III	6	Er:YAG laser	1 x 2 4 x 1	2 lower central incisors 2 lower lateral incisors 1 upper central incisor 1 upper lateral incisor
IV	6	CO_2 laser	2 x 2 2 x 1	3 lower central incisors 2 upper central incisors 1 upper lateral incisor
V	6	Er:YAG laser + NaF 2%	2 x 2 2 x 1	2 lower central incisors 1 upper central incisor 1 upper lateral incisor 1 lower lateral incisor 1 lower lateral canine
VI	6	CO_2 laser + NaF 2%	2 x 2 2 x 1	3 lower lateral incisors 2 upper central incisors 1 lower central incisor

4.3. Tooth Preparation

Following local anesthesia, forceful scaling of the examined surface was performed using hand instruments[1] followed by application of 1% citric acid by means of a cotton swab for 1 min to ensure the removal of the smear layer and expose the dentinal tubules (Figures 1a-c) (51, 67). Then, examined surface was washed with distilled water. Citric acid was freshly prepared by mixing citric acid powder with distilled water within 48 hr before usage.

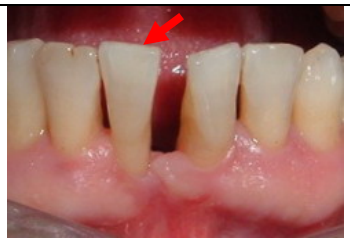
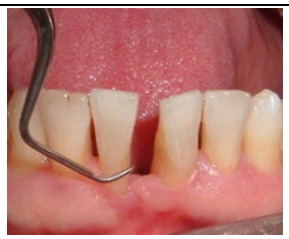
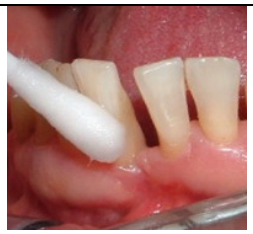
		

Figure 1a	**Figure 1b**	**Figure 1c**

Figure 1a: Arrow shows the tooth indicated for extraction.
Figure 1b: Forceful scaling
Figure 1c: Citric acid application

Group I served as control and no further treatment was applied to the teeth. In group II, NaF 2% (neutral pH) was applied to the dried surfaces using disposable brushes and was allowed to cover the tooth surface for 2 min (Figure 2). In group III, dentinal surfaces were lased by Er:YAG (Figures 4a,b) whereas in group IV the teeth were lased by CO_2 (Figures 4c,d) laser. In groups V and VI, teeth surfaces were treated with combined NaF 2% gel and Er:YAG or CO_2 lasers, respectively.

[1] **Gracey**, Hu-Friedy Mfg. Co., Inc., USA

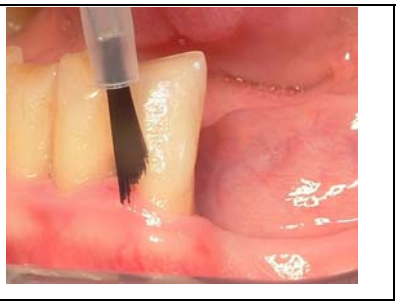


Figure 2: NaF 2% gel application

After completing the selected treatment, the tooth was extracted atraumatically in a manner not to touch the examined surface and not to allow blood to reach it (Figures 3a,b). If traumatization of the prepared surface by the forceps occurred during extraction, the tooth had to be excluded from the study. Immediately after extraction, teeth were washed with distilled water. Then teeth were stored in 4 ºC distilled water and thymol till the experiment.

Figure 3a

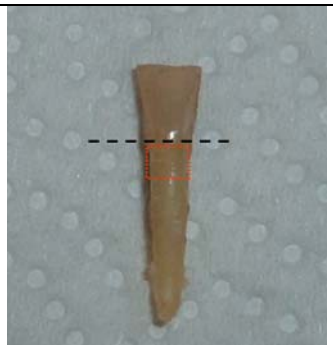


Figure 3b

Figure 3a: Tooth extraction.

Figure 3b: Extracted tooth: Red square shows the prepared area of the root to be examined by SEM; horizontal black line represents the cemento-enamel junction.

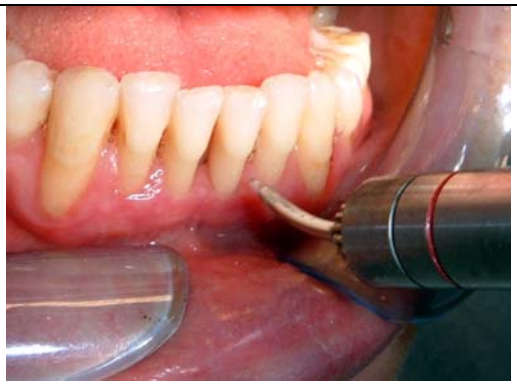
4.4. Laser Application

An Er:YAG laser device[2] was used to deliver a 2.94 μm wavelength beam with a 30° curved quartz round tip of 1200 μm diameter. The laser beam was swept in a mesiodistal fashion for approximately 15 sec with the beam directed perpendicular to the dentin surface in non-contact mode. Energy output of 50 mJ per pulse with pulse duration of 250 msec and frequency of 3 Hz with water coolant was used. The Er:YAG laser device and the delivery through the hand piece to the tooth surface are shown in Figures 4a,b, respectively.

The CO_2 laser[3] with wavelength 10.6 μm was delivered with a hollow tube with a right angle hand piece. A continuous wave with power output of 0.5 W was used, and the laser beam was applied perpendicular to the dentinal surface in a mesiodistal fashion for approximately 30 sec until the entire target surface has been lased. Tip of hand piece was held 2-3 mm away from tooth surface for non-focused lasing. The CO_2 laser device and the delivery through the hand piece to the tooth surface are shown in Figures 4c,d.

During the use of both lasers, protective eye-glasses were worn by the patient and the operators.

[2] **Versawave**, Hoya ConBio, California, USA.
[3] **MedArt 610**, Intros, Hvidovre, Denmark.

	
Figure 4a	**Figure 4b**

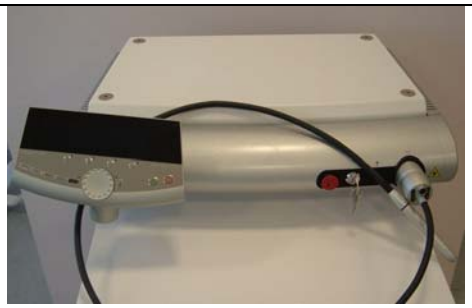
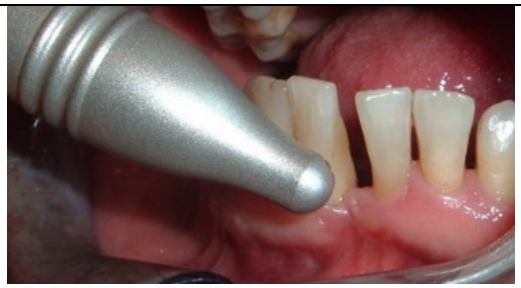

	

Figure 4c	**Figure 4d**

Figure 4a: Er:YAG laser device

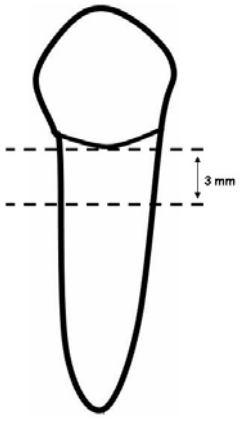
Figure 4b: Delivery of Er:YAG laser to tooth surface

Figure 4c: CO_2 laser device

Figure 4d: Delivery of CO_2 laser to tooth surface

4.5. SEM Examination

A 3 mm thick slice was obtained from the treated tooth surface using a low speed diamond wafering blade[4] (Figures 5a-c). Two transverse cuts were made to prepare each slice, one at the cemento-enamel junction and the second approximately 3 mm apical to the first one (Figure 5b). A third longitudinal cut passed through the root canal in a plane parallel to the dentin surface to be examined (Figure 5c). Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffered saline (pH 7.2) for 24 hr at room temperature, washed out with distilled water and critical-point dried.



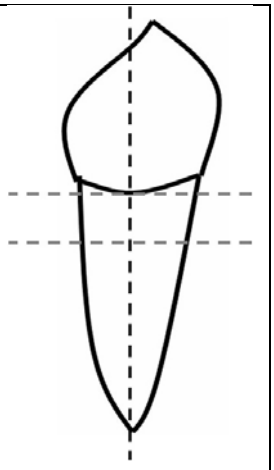


Figure 5a	Figure 5b	Figure 5c

Figure 5a: Low speed diamond wafering blade

Figure 5b: Buccal illustration of a lower premolar: two horizontal cuts; one at cemento-enamel junction and the other 3mm apically to it

Figure 5c: Proximal view of same tooth to present third vertical cut

[4] **IsoMet Law Speed Saw**, Buechler, Illinois, USA.

Specimens were then mounted on aluminum stubs using silver paste and marked by code numbers to keep reference (Figure 6a). Coating of the specimens with a thin layer of gold (20 nm) was obtained using an ion sputtering device[5] under vacuum pressure of 10^{-3} torr, voltage 1.0 kV, current 20 mA, for 5 min (Figure 6b). The gold-sputtered specimens (Figure 6c) were examined with SEM[6] and the surface characteristics and dentinal tubular occlusion/patency were evaluated at 5-15 kV with zero tilt angle (Figure 6d).

	
Figure 6a	**Figure 6b**
	
Figure 6c	**Figure 6d**

Figure 6a: Samples mounted on stubs with numeric coding

Figure 6b: Samples after gold coating

Figure 6c: Ion sputter coater

Figure 6d: Scanning electron microscope device

[5] **Edwards S150B sputter coater**, Edwards high vacuum inc., West Sussex, UK
[6] **Jeol JSM 6335F**, Jeol Ltd., MA, USA.

SEM photomicrographs for each specimen were taken at magnification values of x1000 and x2000. The dentin surfaces were examined for general morphological characteristics, number and diameters of dentinal tubule orifices. All morphometrical measurements were performed by a single investigator. When present, the diameters of the dentinal tubule orifices were measured on the photomicrographs and converted to the real size.

4.6. Statistical Analysis

The means and standard deviations of the measurements were determined. The means of numbers of open dentinal tubules per 100 μm^2 for each group was also calculated from the x1000 magnification photomicrographs. The statistical analysis was performed using a commercially available GraphPad Prisma V.3 software program[7]. Besides standard descriptive statistical calculations, Kruskal Wallis test was used in the multiple comparison of the groups, post Hoc Dunn's multiple comparison test was utilized for intra-group comparisons. Statistical significance level was established at $p<0.05$.

[7] **GraphPad Prisma V.3**, GraphPad Software Inc, San Diego, USA.

5. Results:

5.1. Group I

The photomicrographs of the samples in group one showed clearly exposed dentinal tubules with a complete removal of the smear layer and cementum (Figure 7). The tubules appeared as tunnels that are parallel to each other and with varying diameters (Figure 8) (Table 3).

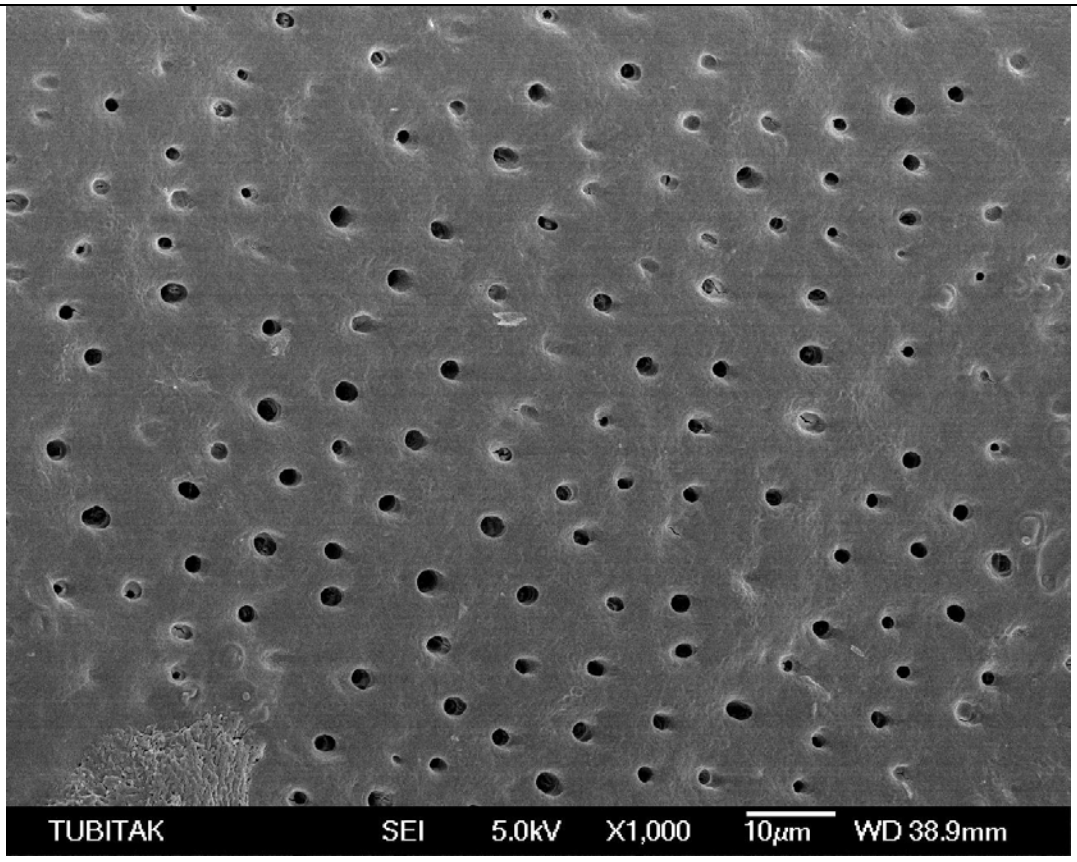


Figure 7. Morphology of dentin surface prepared with citric acid only. Notice the complete exposure of the dentinal surface showing the entries dentinal tubules (Mag. x1,000).

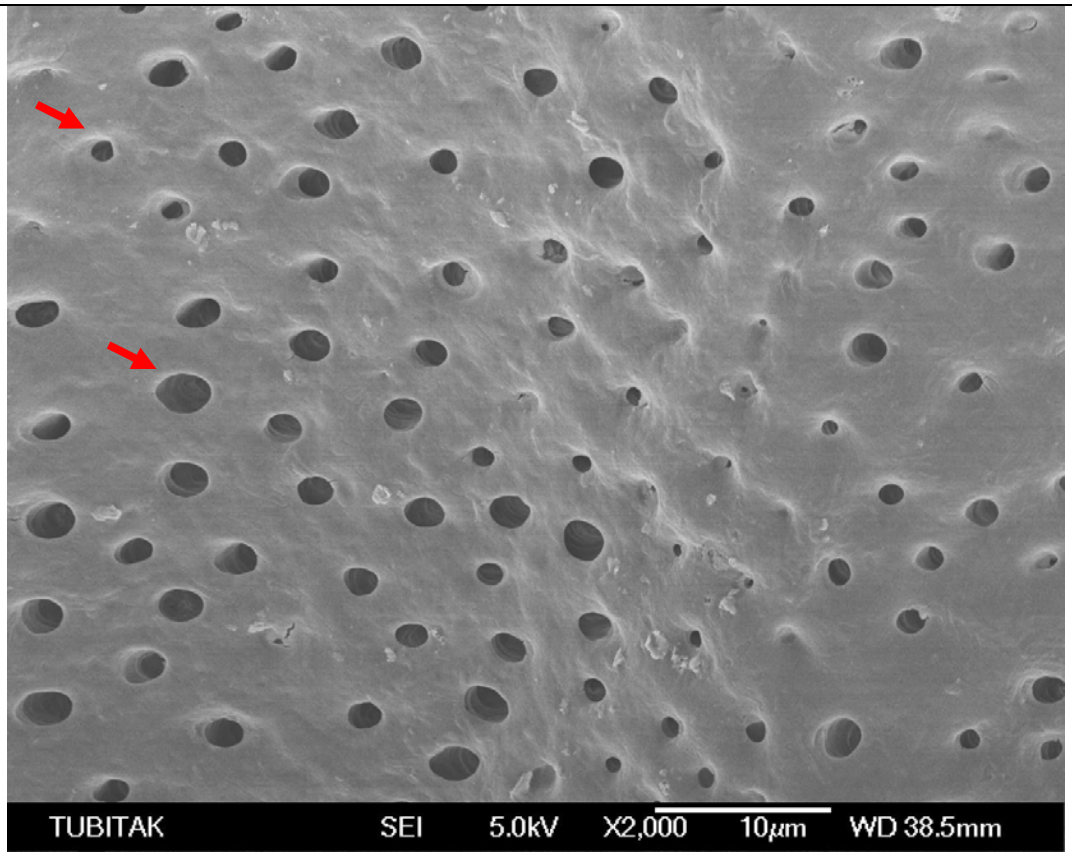


Figure 8. Morphology of dentin surface prepared with citric acid only. Arrows show dentinal tubules with varying diameters (Mag. x2,000).

5.2. Group II

The dentinal tubules seemed partially obturated (Figure 9). The entries of the opened dentinal tubules showed a funnel-like narrowing towards the inside of the tunnel and away from the surface. At the end of the funnel a split-like free entrance remained (Figure 10).

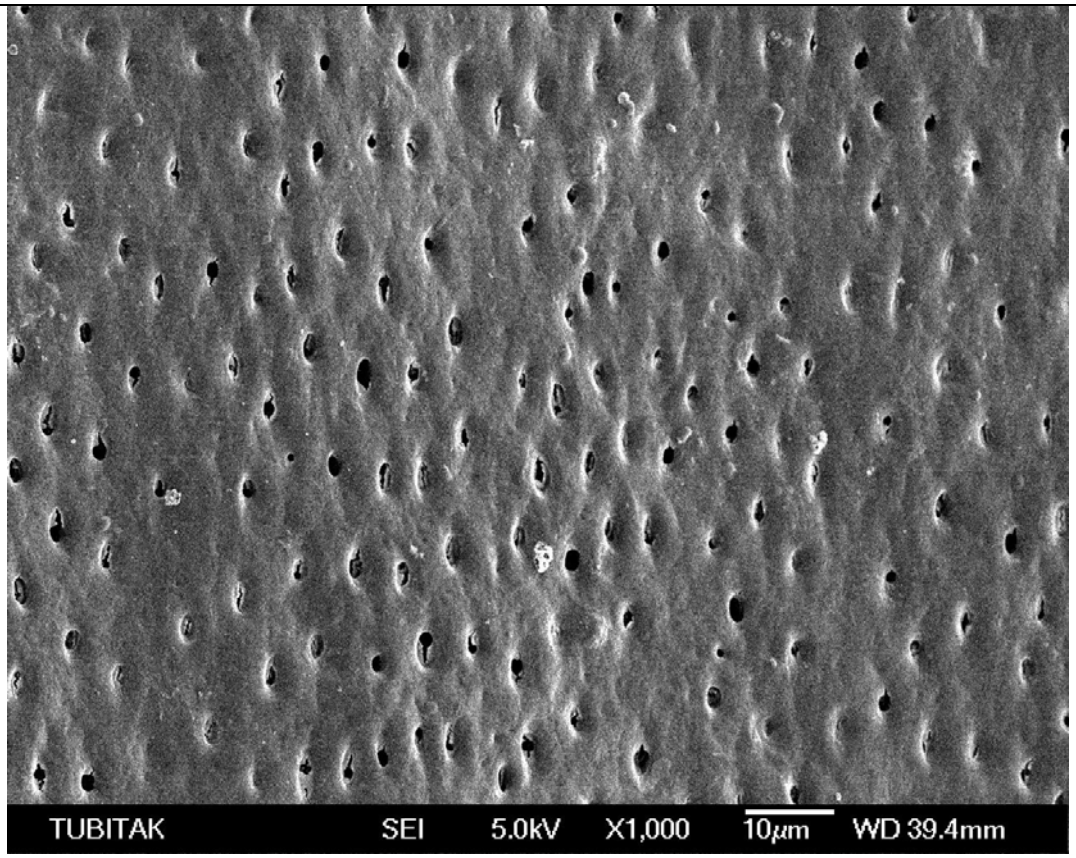


Figure 9. Morphology of dentin surface treated with 2% NaF gel (Mag. x1,000).

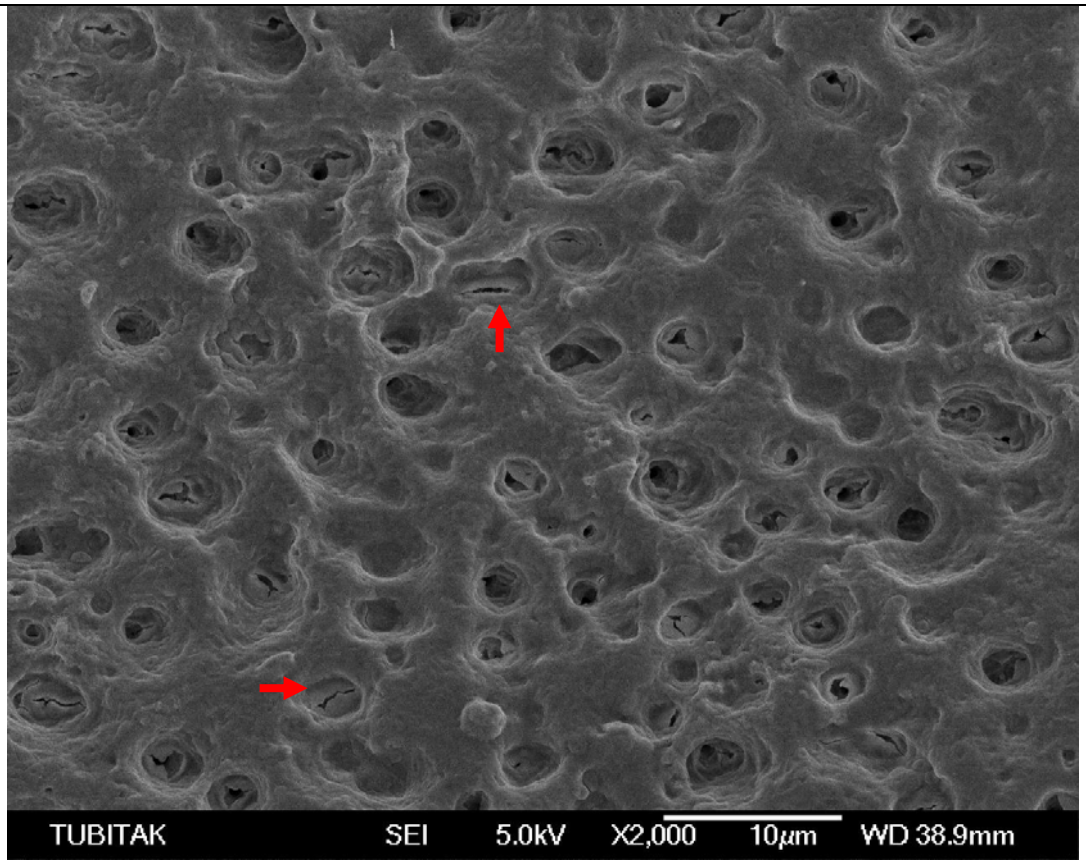


Figure 10. Morphology of dentin surface treated with 2% NaF gel. Arrows show funnel-like narrowed tubules (Mag. x2,000).

5.3. Group III

The surface of the root seemed smooth with the rare presence of open tubules (Table 4), also a crater protrusion of the tubular openings was observed (Figure 11). The morphology of the surface seemed to be a result of melting of the tooth surface which produced a fibrillar appearance after cooling down (Figure 12).

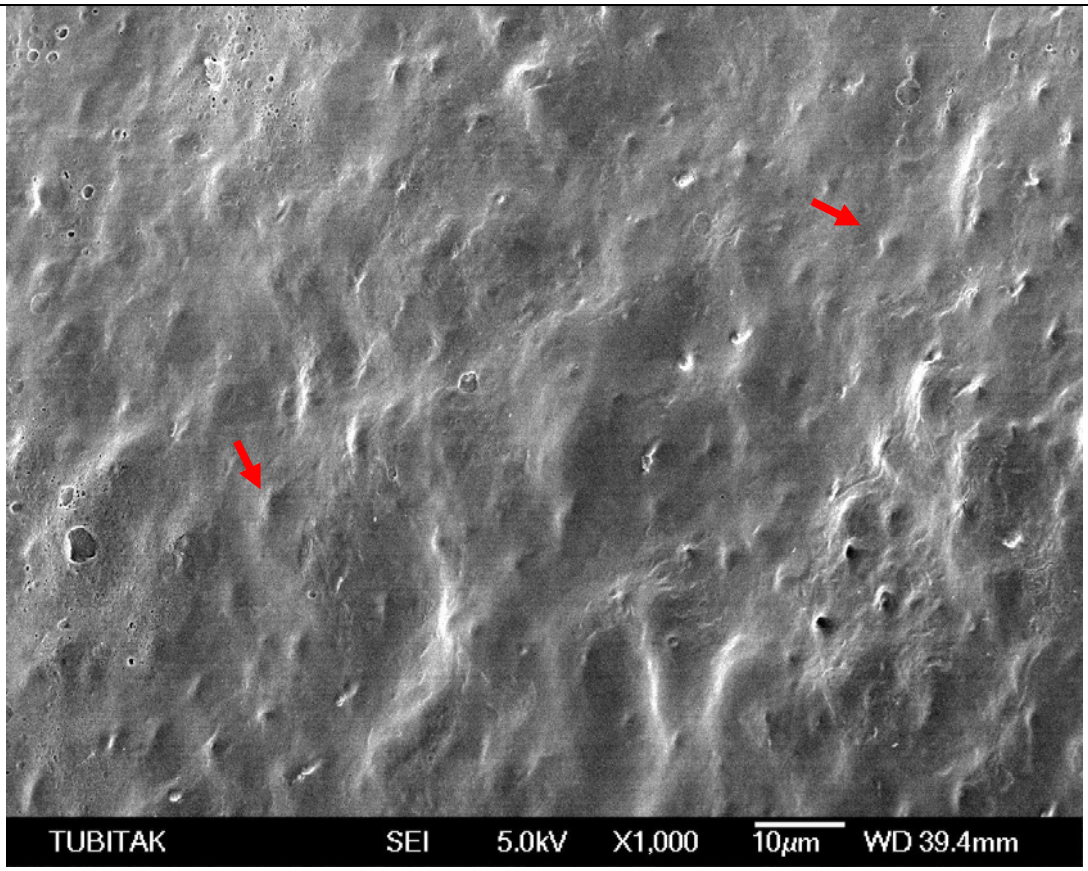


Figure 11. Morphology of dentin surface treated with Er:YAG laser. Notice the melted appearance of the dentinal surface. Arrows show crater protrusions of the tubuler openings (Mag. x1,000).

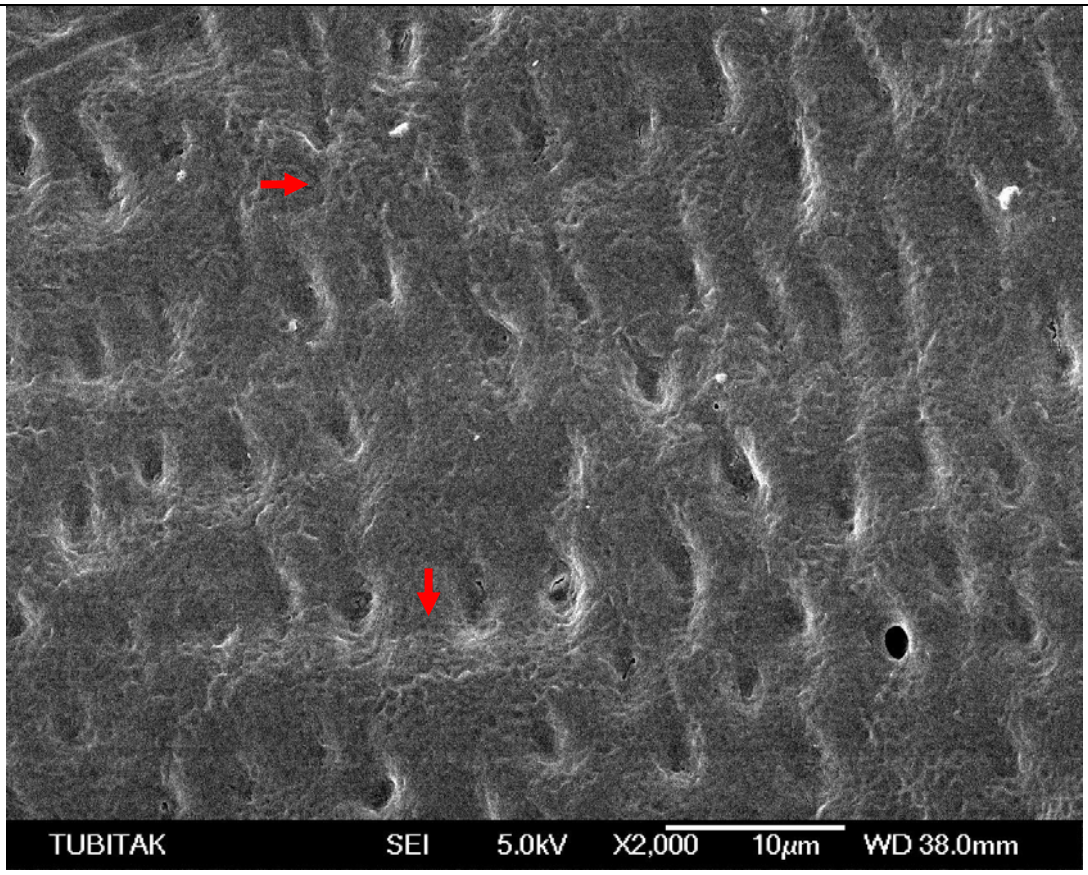


Figure 12. Morphology of dentin surface treated with Er:YAG laser. Arrows show fibrillar appearance formed by cooling down after melting (Mag. x2,000).

5.4. Group IV

Closure of the majority of the openings of dentinal tubules has been obtained (Table 4). The remaining open tubules have narrow split-like entries. A high melting appearance has caused a change of surface appearance giving a whirl-like structure around each supposed dentinal tubule entry (Figure 13).

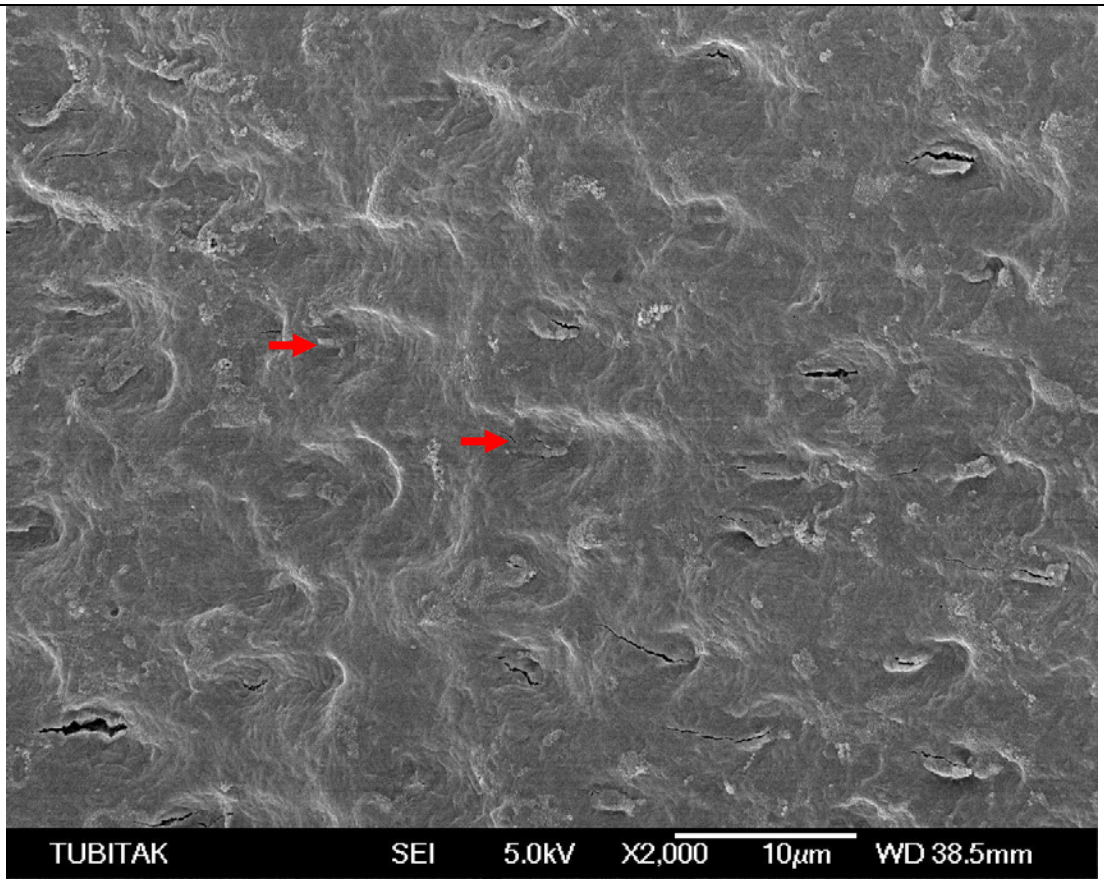


Figure 13. Morphology of dentin surface treated with CO_2 laser. Arrows show the whirl-like structure around the supposed dentinal tubules (Mag. x2,000).

5.5. Group V

The specimens displayed melted surface structures and the entries of the supposed dentinal tubules showed slight elevations like small hillocks (Figure 14). Entries of the present dentinal tubules were partially obstructed (Figure 15). The general surface overview was an irregular melted and cooled shiny amorphous structure.

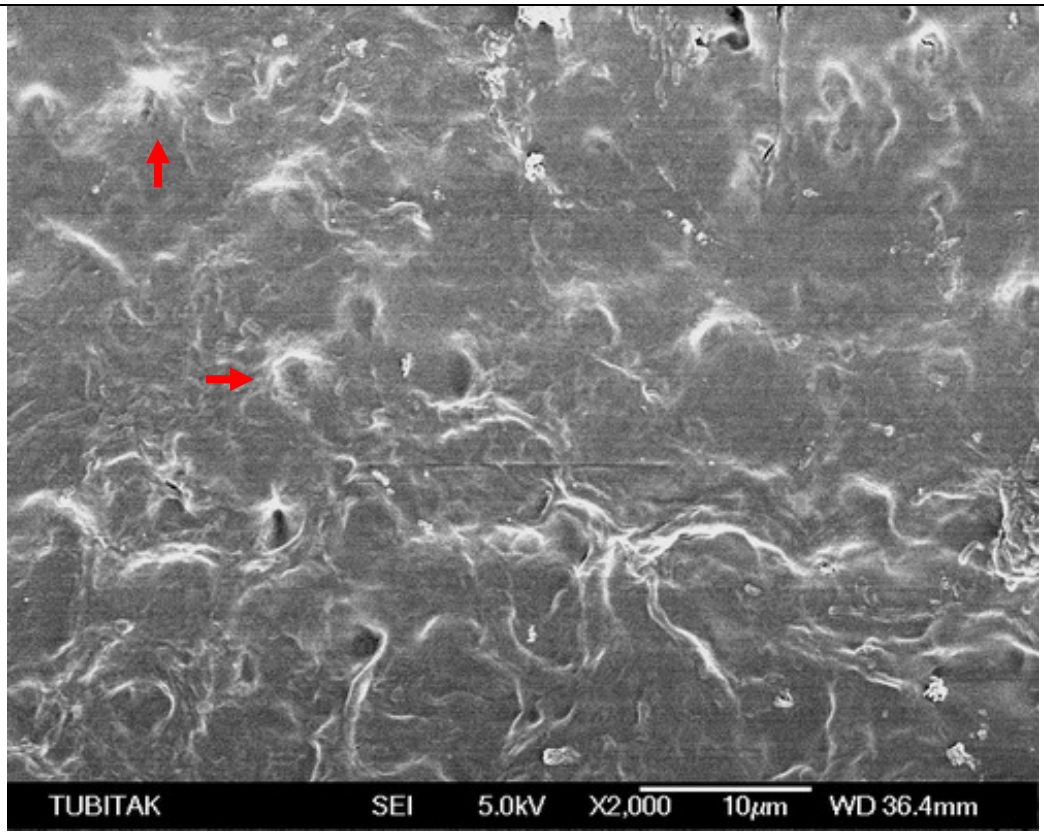


Figure 14. Morphology of dentin surface treated with 2% NaF gel and Er:YAG laser. Arrows show small hillocks elevations at the supposed dentinal tubules (Mag. x2,000).

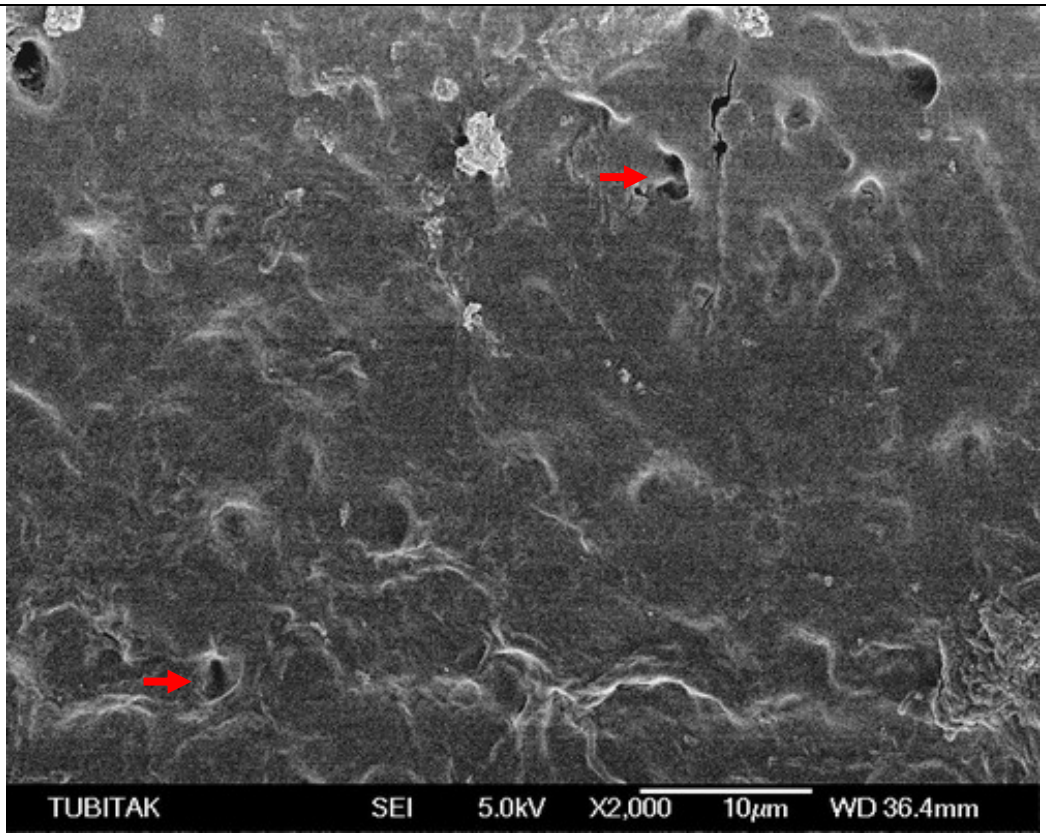


Figure 15. Morphology of dentin surface treated with 2% NaF gel and Er:YAG laser. Arrows show partially obstructed tubules (Mag. x2,000).

5.6. Group VI

Complete closure of the dentinal tubules has been achieved by the formation of an irregular melted amorphous surface. In some localizations, the impression of superposed melted layers is observed (Figure 16). The locations of the few tubules observed show a depression at the tubular entry like a funnel narrowing (Figure 17).

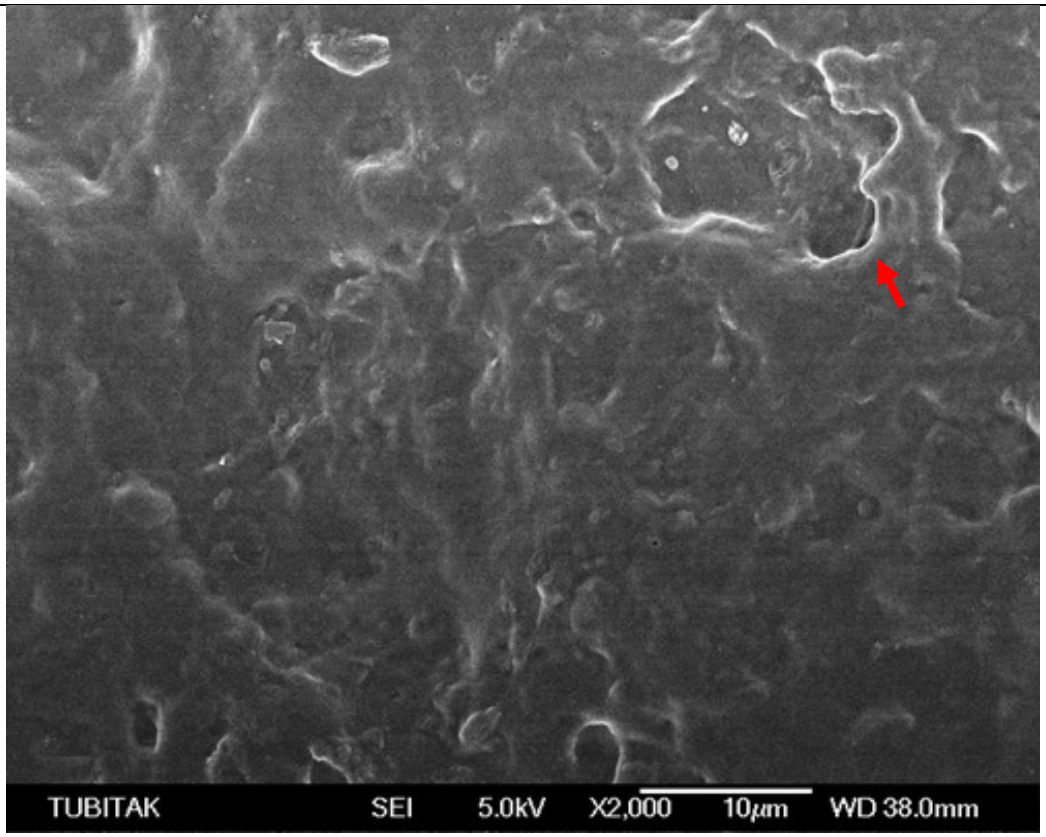


Figure 16. Morphology of dentin surface treated with 2% NaF gel and CO_2 laser. Notice the complete closure of the dentinal tubules. Arrow shows locations of superposed melted layers (Mag. x2,000).

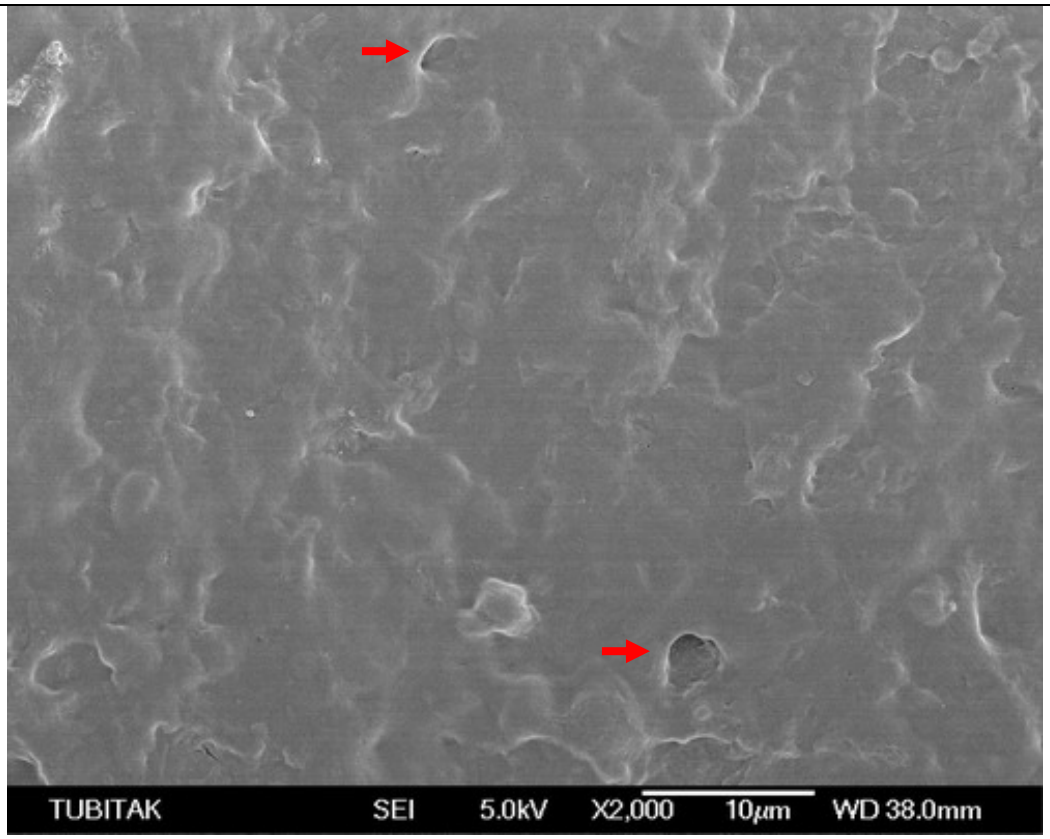


Figure 17. Morphology of dentin surface treated with 2% NaF gel and CO_2 laser. Notice the irregular melted surface. Arrows show depression at the tubular entry like a funnel narrowing (Mag. x2,000).

5.7. Measurements

As shown in Table 3, the diameters of the open tubules were the largest in group I (mean 1.96±0.60 μm) and lowest in group V (0.38 ±0.60). There was a statistically significant difference between the groups when Kruskal Wallis test was used ($p<0.01$, Table 3). The number of open dentinal tubules was highest in group I (mean 147.8±12.07) and lowest in group VI (mean 0.50±0.84) as shown in Table 4. For the number of open dentinal tubules, Table 4 reveals inter-group statistically significant difference using Kruskal Wallis test ($p<0.001$). Results of Dunn's multiple comparisons are shown in Table 5 for the diameter of dentinal tubules values and number of open tubules per 100 μm^2 values. For diameters of dentinal tubules, the results revealed statistical significant difference between groups I and V ($p<0.05$) and groups I and VI ($p<0.05$). For numbers of open dentinal tubules, group I showed statistical significant difference compared to the groups III, IV, V, and VI, also group II showed statistical significance compared to groups III, IV, V, and VI (Table 5).

Table 3. Mean values and medians values of diameters of dentinal tubules (μm) and comparison of all groups.

Sample code within group	a	b	c	d	e	f	**Mean± SD**	**Median**
Group I	2.23	1.81	2.55	2.62	1.34	1.22	**1.96 ±0.60**	**2.02**
Group II	1.36	1.17	0.87	1.04	0.76	1.57	**1.12 ±0.30**	**1.1**
Group III	0.90	1.03	0.0	0.0	1.04	0.74	**0.62 ±0.49**	**0.82**
Group IV	0.54	1.57	1.17	0.0	0.0	0.0	**0.55 ±0.68**	**0.27**
Group V	1.35	0.90	0.0	0.0	0.0	0.0	**0.38 ±0.60**	**0.0**
Group VI	0.67	0.0	0.0	0.0	1.98	0.0	**0.44 ±0.80**	**0.0**
							KW:16.43 p<0.01	

Table 4. Number of open dentinal tubules

Sample code within group	a	b	c	d	e	f	**Mean± SD**	**Median**	**Number of open tubules per 100 µm^2**
Group I	131	159	147	136	143	161	**147.8±12.07**	**145.0**	**1.19**
Group II	117	110	130	105	114	119	**115.83±8.56**	**115.5**	**0.94**
Group III	3	1	0	0	2	3	**1.50±1.38**	**1.5**	**0.01**
Group IV	7	4	3	0	0	0	**2.33±2.87**	**1.5**	**0.02**
Group V	3	2	0	0	0	0	**0.83±1.32**	**0.0**	**0.007**
Group VI	1	0	0	0	2	0	**0.50±0.84**	**0.0**	**0.004**
							KW:26.86 p<0.001		

Table 5. Inter-group comparisons.

Dunn's Multiple Comparison Test	**Diameter of dentinal tubules (µm)**	**Number of open tubules per 100 µm^2**
Group I / Group II	P > 0.05	P > 0.05
Group I / Group III	P > 0.05	**P < 0.01**
Group I / Group IV	P > 0.05	P < **0.05**
Group I / Group V	**P < 0.05**	**P < 0.01**
Group I / Group VI	**P < 0.05**	**P < 0.001**
Group II / Group III	P > 0.05	**P < 0.05**
Group II / Group IV	P > 0.05	**P < 0.05**
Group II / Group V	P > 0.05	**P < 0.05**
Group II / Group VI	P > 0.05	**P < 0.01**
Group III / Group IV	P > 0.05	P > 0.05
Group III / Group V	P > 0.05	P > 0.05
Group III / Group VI	P > 0.05	P > 0.05
Group IV / Group V	P > 0.05	P > 0.05
Group IV / Group VI	P > 0.05	P > 0.05
Group V / Group VI	P > 0.05	P > 0.05

6. Discussion and Conclusion

Lasers are one of the promising new technical modalities used for the treatment of dentinal hypersensitivity. Various lasers have been used for this purpose including Nd:YAG, Er:YAG, CO_2, and diode laser (54, 95, 107, 112). Among these, the use of Er:YAG and CO_2 is promising due to the properties of these lasers which can modify dentinal surfaces without harmful effects on the pulp or surrounding tissues. The desensitizing potential of these lasers have been investigated in limited studies, also no previous report has compared the morphological effects of NaF, Er:YAG laser, and CO_2 laser on dentinal tubules. Therefore, the aim of this study was to evaluate the qualitative potential of Er:YAG and CO_2 laser with or without 2% NaF to occlude the open dentinal tubules which are believed to associate and cause dentinal hypersensitivity.

This study was designed as an *in vivo* SEM analysis where surface morphological effect of the used therapies can aid the investigator to create the knowledge to assist choosing the appropriate treatment. It is well established that exposure of open dentinal tubules is related to dentinal hypersensitivity. According to the suggested theories of pain transmission, dentinal tubules play a key role in passing the stimulus from the tooth surface to the pulp. Clinical studies have showed that hypersensitivity associates the presence of exposed dentinal tubules on the tooth surface (1, 89). Factors such as number and width of these tubules are proportional to the severity of the stimulus delivered to the pulp (1, 3). Therefore, occlusion or narrowing of the tubules seems to be the desired outcome of a successful desensitizing therapy. The methodology of this study assists to examine the true *in vivo* effect of Er:YAG and CO_2 lasers used alone and in combination with NaF on the morphology of the root surface.

This study required root surfaces with exposed dentinal tubules, which should be isolated during and after treatment until special preparation for SEM. In the literature citric acid and scaling have been reported as the preferred methods to expose dentinal tubules (51). Thus, forceful scaling was performed using hand instruments to remove any remaining

cementum and freshly prepared citric acid was used to eliminate the remnants of naturally-occurring smear layer. McAndrew and Kourkouta (67) have claimed that the number and diameter of open tubules resulting from application of 1% citric acid for 1 min resembles clinically sensitive dentin.

The extraction procedure was carried on using conventional forceps, but the grasp was limited to the anatomical crown to avoid any contact of the forceps to the prepared area which could cause trauma and destroy the surface to be examined by SEM. This was also done to ensure the complete isolation of the tooth so that it won't be contaminated by blood or saliva. The tooth had to be discarded and removed from the study if such contamination was observed.

During the process of the experiment, none of the patients felt any discomfort or complications different than normal extraction procedures. The laser application was of short duration and caused no pain to the patient. No deformation to the tooth was observed by the naked eye. Also there was no production of smell or sparks during the application of both lasers.

The results from group I revealed that the proposed method to expose the openings of dentinal tubules was appropriate. The microphotographs showed a clear smooth surface of intertubular dentin with well ordered and uniformed dentinal tubules typical of views showed in other human dentinal studies (Figure 5). The diameters of dentinal tubules had an average of 1.96 ±0.60 μm (Table 3) and the number of open dentinal tubules was 1.19 per 100 μm^2 (Table 4). Isik et al. (42) reported the diameter of dentinal tubules to be 1.76-2.12 μm on control samples which was increased up to 4.60 ±1.54 μm when washed for 1 minute with a concentration of 50 mg/ml of tetracycline hydrochloride. Kumar and Mehta (51) observed tubules with diameters of a range of 2.98-4.65 μm after the exposure to 1 % citric acid. Misra et al. (71) used saturated citric acid for 3 minutes and found the diameters of dentinal tubules of 4.39 ± 0.93 μm, they also reported the presence of 0.99±0.18 open dentinal tubules per 100 μm^2. Using acids, some amount of decalcification of the dentinal surface occurs; this would enlarge the openings of the

dentinal tubules during preparation of tooth and cause the variations between different studies (42).

In Group II, 2% NaF application to the root surface had narrowed the diameters of the dentinal tubules to 1.12 ±0.30 µm compared to 1.96 ±0.60µm in group I (Table 3). Also the average of the numbers of dentinal tubules was less in group II (0.94/ 100 μm^2) compared to (1.19/ 100 μm^2) in group I (Table 4). However, these differences are not statistically significant ($p>0.05$, Table 5). This decrease is due to the reaction of NaF with dentin and precipitation of calcium fluoride in the dentinal tubules (28, 55). Similar results have been reported where a reduction of the diameters of dentinal tubules from 4.13 ±0.49 µm to 1.93 ±0.17 µm occurred after the application of 5% NaF varnish to dentinal surface in an *in vivo* study (51). Previous SEM studies have also investigated the effect of fluoride containing compounds on the dentinal surfaces of extracted teeth. The results were comparable between the studies were a tubule closure range of 40-80% had been reported with the use of fluoride containing compounds. The authors observed granular structures on the surface of dentine and lumen of the dentinal tubules in their photomicrographs (9, 88). This higher occlusion rate in comparison to our study might be attributed to the repeated application of fluoride compounds (twice a day for seven days). Gaffar (27) stated that treatment with fluoride forms a protective layer of calcium fluoride that prevents fluid flow, thereby reducing dentinal hypersensitivity. This effect has been well demonstrated clinically, where several studies showed that fluoride has been capable to reduce or eliminate hypersensitivity immediately and for periods of time reaching up to 6 months (41, 51, 63, 79, 98).

In group III, the photomicrographic image of the smooth surface demonstrates the blockage of the openings of dentinal tubules. Samples treated with Er:YAG laser had significant less number of open dentinal tubules compared to group I ($p<0.01$, Table 5) and group II ($p<0.05$, Table 5). The diameters of the remaining open dentinal tubules had a mean of 0.62 ±0.49 µm (Table 3) which was smaller but not statistically significant compared to group I ($p>0.05$, Table 5). Er:YAG beam is readily absorbed by water, causing an evaporation of the dentinal fluid. This results in the deposition of the insoluble

salts in the dentinal tubules and obturating them. Previously, Schwarz et al. (95) have clearly showed that Er:YAG laser (80 mJ, 3 Hz) was able to reduce symptoms of hypersensitivity in a clinical follow-up study up to 6 months. They suggested that effect of laser may not only be contributed to the evaporation of dentinal fluid, but also to its high bactericidal effect which would reduce the inflammatory mediators resulting in a higher pain threshold. Watanabe et al. (107) have also reported reduction of dentinal hypersensitivity after irradiation of 5-10 mJ/pulse and 10 Hz. According to their SEM analysis, they suggested that evaporation of the water content lead to degranulation or coagulation of the organic elements causing their accumulation and blockage of dentinal tubules.

According to the photomicrographs in this study, 2% NaF addition gave better results as seen in group V compared to the Er:YAG alone therapy in group III. Resulting surface structure is different in group V which is amorphous; this appearance gives an impression that this structure is harder and less vulnerable to environmental factors than that of group III. The diameters of dentinal tubules in group V were significantly less in comparison to group I ($p<0.05$, Table 5), whereas comparisons of group III versus group I didn't show statistical significance ($p>0.05$, Table 5). The presence of a thin layer of NaF 2% provides the reactive fluoride ions which would rapidly engage in newly formed salts, resulting in a layer that would block the surface of dentinal tubules. Similar to our observations, NaF has also been reported to be useful when combined with Nd:YAG laser in the management of hypersensitivity (51, 55). In an *in vitro* SEM study, Lan et al. (55) found that most dentinal tubules orifices were occluded after the combined treatment of Nd:YAG laser with NaF varnish. They found that the formed surface was resistant and remained after electrical toothbrushing for 30 min. For the NaF alone group, closure was obtained after application, but tubules were reopened after electrical brushing. The authors suggested that Nd:YAG laser causes melting of the dentine and closure of dentinal orifices without producing dentine surface cracks. Later on, Lan et al. (54) conducted an *in vivo* study, and evaluated the surfaces using impressions which were evaluated by SEM. They found that Nd:YAG laser was able to seal dentinal tubules in accordance to their previous study (55). They proposed that the mechanism of Nd:YAG

laser's effect on dentine is caused by thermal energy absorption. The thermal energy generated by the laser was quickly absorbed by the dentine causing melting of the hydroxyapatite crystals which are moved and increased in size once the activation energy was sufficient (54).

Kumar and Mehta (51) evaluated the efficiency of Nd:YAG laser irradiation *in vivo* alone and in combination with 5% NaF varnish in the management of dentin hypersensitivity using SEM. Teeth were extracted immediately after treatment and clinical evaluation of hypersensitivity. They reported that the number and diameters of open tubules significantly decreased after both laser alone or combined with NaF compared to untreated samples. However, in the Nd:YAG and NaF combination group, most of the tubules were closed and the diameters were significantly less than either the laser alone or NaF alone groups. Clinical symptoms were also in accordance with the SEM observations; hypersensitivity was most significantly released in the combined treatment group where pain was reduced by 62%. In the 2% NaF alone and Nd:YAG alone groups, symptoms were reduced by 33% and 44%, respectively. The authors concluded that the combination of Nd:YAG laser and 5% NaF varnish seems to show an impressive efficacy in treating dentin hypersensitivity. The SEM findings seemed to relate to the clinical findings in that reduction in number and patency of tubules was associated with improvement in treatment efficacy (51). These observations are also consistent with the findings of a clinical study by Liu and Lan (60). They claimed that the combined use of laser with NaF application enhanced treatment effectiveness by more than 20% over that of the laser alone group. The SEM observations in this study are comparable to those given by Kumar and Mehta (51), the photomicrographs of groups III and V presented a superior view compared to their photomicrographs. Therefore, it can be speculated that the actual elimination of hypersensitivity using Er:YAG laser alone or combined with NaF may be accomplished in a rate similar to the mentioned study.

To develop a therapeutic modality for hypersensitivity, Grossman (36) indicated that such treatment should have no harmful effect on the tooth. Excessive laser irradiation may cause serious damage to the root surface and the pulp. Schwarz et al. (96) examined the

effect of Er:YAG laser on root surface using SEM. They used energy settings ranging from 120 - 180 mJ at 10 Hz. These settings were applied to root surfaces under *in vivo* and *in vitro* conditions. They observed no morphological destruction in the *in vivo* group; the produced surfaces were homogenous and smooth with no signs of thermal damage. Also the results did not seem related to the used energy setting. On the other hand, they reported that the *in vitro* application produced ablation of tooth substance with chalky notches and deep craters. These results demonstrate that the effect of Er:YAG laser is much less harmful when used *in vivo* than *in vitro*. Later on, Schwarz et al. (97) evaluated root surfaces after irradiation with 160 mJ at 10 Hz under water irrigation in an *in vivo* attempt to selectively remove calculus. Using light microscopy, they found that laser had no harmful effect such as carbonization or cracking on the root surface resulting in a smooth surface. Furthermore they were successfully able to remove the calculi. Concerning the temperature of the pulp, Theodoro et al. (102) conducted an *in vitro* study where Er:YAG laser at 100 mJ and 10 Hz for 30 sec with water coolant was applied to root surfaces of extracted teeth after scaling. The temperature was monitored by means of a type T thermocouple (copper-constantan) positioned in the pulp chamber to assess pulpal temperature during and before irradiation. Thermal analysis showed that the temperature of the teeth after irradiation was lower than the temperatures registered before lasing; temperature change was -2.2 ±1.5 °C and this was contributed to the effect of water coolant. Overall, the aforementioned studies show that the energy setting used in this study, which was 50 mJ at 3 Hz with water coolant, was lower than the safety limits and could not cause damage to the tooth surface or the pulp.

In group IV, decrease in number of open dentinal tubules was observed on the photomicrographs after CO_2 laser therapy (0.02 tubules/ 100 μm^2, Table 4), this difference was statistically significant compared to groups I and II ($p<0.05$, Table 5). The remaining open tubules had narrow split-like entries with mean diameters of 0.55 ±0.68 μm (Table 3) which was not statistically different compared to any other group (Table 5). This resulting appearance seems to be due to melting of the dentinal substance which formed a new structure during cooling down, eventually obturating the open dentinal tubules. These SEM results are comparable to studies which investigated the usage of

CO_2 laser on root surfaces to enhance healing results of periodontal therapy. Barone et al. (10) applied CO_2 laser to freshly extracted teeth. Two laser settings were used: group A, 8.0 W in focused continuous wave; group B, 2.0 W in non-focused pulsed wave of 4 Hz. Group A exhibited zones of heat cracking, fissuring, and pronounced roughness under SEM examination; this was obviously due to the high power settings used. However, in group B dentinal tubules were completely sealed, and the dentin appeared as a shiny melted layer showing a flat and smooth surface. Treatment settings in group B are more close to the settings used in this present study; moreover, the resulting surface is much similar. When 2% NaF was applied to the root surface before application of CO_2 laser in group VI, the surface seemed more contact with almost complete absence of open dentinal tubules (0.004 tubules / 100 μm^2, Table 4). The melted layer may be thicker in group VI where fluoride has contributed to the new surface structure. The microphotograph appearances suggest that hypersensitivity should have been reduced in both groups IV and VI due to the occlusion of the dentinal tubules, there is no statistical difference between both groups in manners of diameters of dentinal tubules or number of open dentinal tubules ($p>0.05$, Table 5). However, from the microphotographs, combined treatment of CO_2 laser and 2% NaF gel (group VI) seems to form a more contact surface layer which may be more capable of reducing hypersensitivity.

Clinically, Moritz et al. (74, 76) showed that the combined CO_2 laser irradiation (0.5 W in continuous mode) and fluoridation was able to reduce hypersensitivity in 96.5% of the examined patients for a period of 18 months. This was significantly more effective than the control group which received fluoridation only. Atomic absorption spectroscopy was also performed on dentinal samples obtained from dental necks at 6 weeks and 18 months after laser therapy; it showed the presence of Tin indicating permanent integration of fluoride with dentin. Two teeth were examined using SEM at 4 and 6 months after lasing, they revealed complete closure of the dentinal tubules (76). Authors suggested that CO_2 should be considered an ideal tool for desensitizing dental necks. Laser Doppler measurements of pulpal blood flow before and after laser showed no effects of CO_2 laser on pulpal blood flow (74). The power settings used in this study is similar to those used in Moritz et al's study (74, 76) and less than those used in the report by Barone et al. (10).

No damage was detected in any of our samples in confirmation of these studies. Also the closure of the dentinal tubules suggests the possible clinical success of CO_2 laser alone or in combination with NaF.

A general obstacle slowing the improvement of laser research in all dental fields is the fact that the quantity of laser energy applied to a specific target is not easy to be standardized due to the usage of different laser devices. Different systems for production of laser beam have their own available parameters which vary from one system to another in the manner of power output scales, beam energy per pulse, frequency of intermittent beam, and pulse duration. Each system has its own control parameters which are chosen by the manufacturer; where the variance is that each manufacturer has its own style and view. Another variance is the delivery systems and hand pieces which may have an effect on the delivered power and the density of laser beam. Investigators use treatment protocols compatible with the setting parameters provided with their laser devices; and so, comparison of these reports is sometimes complicated. The standardization of laser setting is recommended, research would be much more efficient if such system is present. Such standardization needs to be carefully planned and set by an international organization and encourage investigators to follow it so that conducted procedures can be carried on and improved by following researchers.

Unavoidable, an overall strong placebo effect is commonly described in clinical dentin hypersensitivity trials. This can lead to misleading conclusions in laser treatment trial, where the laser usage has a strong psychological influence on the patient as this technology is escorted with the superstitions of having magical effects. Investigators have observed patients having relief without any treatment due to the placebo effect. This effect is thought to vary from 20 to 60% in dentin hypersensitivity clinical trials (108). The possibility of a placebo effect must be taken into consideration during planning and criticizing a clinical study so that its results won't delude the progress of research.

An important consideration in building such treatment techniques is the applicability and accessibility to the tooth surface. The teeth surfaces are curved and may not be easy to

access due to the surrounding tissues. For this concern, CO_2 laser is more practical due to its easier application to the surface compared to Er:YAG laser. When applied in non-focused mode as used in this study, the continuous CO_2 laser beam works on a relatively large area of the tooth surface (approximately 2 mm^2). This makes the procedure more time saving and more accurate. On the other hand, Er:YAG laser beam is delivered in through narrow tips (600 μm radius) and is irradiated in intermittent pulses which requires good attention to make sure all the targeted surface has been lased, besides it takes longer time compared to the CO_2 laser application. Although the technique seems to be easier with CO_2, both lasers produce successful results in terms of tubule occlusion.

An overall evaluation of both lasers, either alone or combined with 2 % NaF, reveals a statistically significant difference in the number of open dentinal tubules compared to groups I and II which favors the possibility that hypersensitivity can be effectively reduced by these lasers (Table 5). The diameters of the open dentinal tubules were narrower in the laser treated groups (Table 3) but the differences were not significant in comparison to groups I and II; except for groups V and VI which had statistically significant smaller diameters compared to group I (Table 5). However, these findings do not diminish the effect of lasers on hypersensitivity since the number of these measured open tubules was significantly less and should not be expected to affect the clinical outcomes. Furthermore, complete closure was obtained in 2 samples out of the 6 samples examined in group III; also 3 samples out of 6 in group IV, and 4 samples out of 6 in each of the combination treatment groups (V and VI) showed complete closure of the tubular openings (Table 4). This change on the dentinal surface morphology and the obstruction of dentinal tubules is expected to play an effective role on dentinal hypersensitivity as the relationship between the number and diameter of open dentinal tubules has well been established in previous studies (1, 3, 51, 89).

This SEM study presents a promising potential for both Er:YAG and CO_2 lasers as desensitizing tools for hypersensitive teeth. Both lasers give the photomicrographic image that favors the reduction of hypersensitivity in the examined teeth. Combination of lasers with fluoride products was also successful and fluoride seemed to be merged in the

new surface structure. However, these effects' reflection on hypersensitivity remain theoretical and need to be supported by further comprehensive clinical studies. Whether the advantage of these therapies is sustainable for long-term is yet to be ascertained. Further studies should be continued to reach the best possible therapies in this field as lasers may play an important role in the future therapy of dentinal hypersensitivity.

7. References

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8. Curriculum Vitae

I was born in 1979 in Jerusalem - Palestine. Completed High School education in 1997 and graduated as a Dental Surgeon in 2002 with a B.D.S. degree from Cairo University - Egypt. At September 2002, I was sent, by Al-Quds University - Palestine, to further my education in Periodontology and to attend the Department of Periodontology at the Dental Faculty, Yeditepe University.