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Enhancement of Dental Enamel Microhardness using Er.Cr:YSGG and CO_2 Laser with or without Fluoride application

A Thesis Submitted to the Institute of Laser for Postgraduate Studies, University of Baghdad in partial fulfillment of the requirements for the degree of Master of Science in Laser / Dentistry

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿وَلَوْلَا فَضْلُ اللَّهِ عَلَيْكَ وَرَحْمَتُهُ لَهَمَّت طَّائِفَةٌ مِّنْهُمْ أَن يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّا أَنفُسَهُمْ ۖ
وَمَا يَضُرُّونَكَ مِن شَيْءٍ ۚ وَأَنزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا
لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا﴾

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Dedication

To whom I proud to carry his name, My Father.

Thank you for keeping me going even when I thought I could not.

To the tittle of love, tenderness, and hope.

To whom that have taught me to endure, no matter how the circumstances change, My Mother.

To the big heart who taught me success, patience and the emergence of a passion for knowledge

My Beautiful sisters (Alaa , Esraa and Asmaa)

To baby girl, who is the frolic and joy into my life, (Zahraa)

"My God bless you all "

Ahmed

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Abstract

Background: Caries and its prevention are among the major factors to control tooth damage and caries progression. Lasers can interact with tooth elements which may create some change benefit to acid resistance that could be comparable to fluoride.

Aimes: - The goal of this study was to examine the effectiveness of employing the Er.Cr:YSGG CO2 laser to increase tooth acid resistance with and without acidulated phosphate fluoride .

Materials and methods:- Eighty enamel teeth obtained from first premolar and lower third molar were randomly divided into eight groups (n=10): G1: control group (no treatment) , G2:acidulated phosphate fluoride was applied for 4 minutes using a cotton bud, G3: Er.Cr:YSGG laser at 0.25 W, 20 Hz, 1% water and 10% air ,; G4: Er.Cr:YSGG laser at 0.50 W, 20 Hz, 1% water and 10% air ; G5: CO_2 laser at 1.0 W, energy 10 mj, duration time 10.0 ms, interval 1.0 ms, distance 0.2 mm; G6: Er.Cr:YSGG laser at 0.25 W, 20 Hz, 1% water and 10% air and acidulated phosphate fluoride ; G7: Er.Cr:YSGG laser at 0.5 W, 20 Hz, 1% water and 10% air and acidulated phosphate fluoride ; G8: CO_2 at 1.0 W, energy 10 mj, duration time 10.0 ms, interval 1.0 ms, distance 0.2 mm and acidulated phosphate fluoride. After treatment, the samples were exposed for 10 days pH cycling. The samples were tested for Digital Vickers microhardness on (9.8 N) at the enamel surface on baseline, after laser irradiation and after ph cycling. The surface roughness was also measured before and after treatments. Selected samples of all groups were further evaluated by scanning electron microscopy (SEM) group after laser irradiation. Samples of all groups were selected to test Ca/P ratio using energy dispersive spectroscopy (EDS) at base line, after treatment and after PH cycling. Date were statistically analysis using ANOVA and Tukey HSD tests at (α=0.05).

Results : There was a statistically significant difference in microhardness values between baseline and after treatment assessments in the fluoride, laser, and fluoride + laser groups ($P < 0.05$) microhardness measurements revealed a significant increase. However, as compared to the baseline, the fluoride, laser and (laser and fluoride) groups after PH cycling microhardness find to be higher in fluoride and laser groups while the lowest is in the control group (G1) with significant difference. Non-significant difference in roughness value between base line and after treatment. The SEM showed that there was no sign of carbonization or cracks in the laser groups with some surface modifications

Conclusions: When compared to a control group, acidulated phosphate fluoride (APT) application with Er.Cr:YSGG laser irradiation of 0.5 w, 20Hz, 1 % water, and 10% air or CO_2 laser irradiation at 1 W enhanced enamel surface microhardness and reduced enamel demineralization.

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LIST OF ABBREVIATIONS

Abbreviation	**Meaning**
ADA	American Dental Association
ANOVA	Analysis of variance
Ca	Calcium
Ca/P	Calcium Phosphorus Molar Ratio
CO_2	Carbon dioxide
CW	Continuous wave
EDS	Energy Dispersive Spectrometry
Er.Cr:YSGG	erbium, chromium:Yttrium-scandium-gallium-garnet
Er:YAG	Erbium-doped:Yttrium, Aluminum, and Gernet
H	Hours
HA	Hydroxyapatite
Hz	Hertz (unit of frequency)
J/cm^2	Joule per square centimeter (unit of energy density)
LASER	Light Amplification by Stimulated Emission of Radiation
M	Mol
mJ	Milli Joule (unit of energy)
Nd :YAG	Neodymium doped yttrium–aluminium–garnet
Nm	Nanometer (= 10^{-9} m)
P	Phosphorous
PDT	photodynamic therapy
p- value	Probability
FDA	Food and drug administration
PH	Potential of Hydrogen
PRR	Pulse Repetition Rate
S	Second
SD	Standard Deviation
SEM	Scanning Electron Microscope
Tukey HSD	Tukey Honestly Significant Difference
W	Watt
Wt	Weight

LIST OF SYMBOLS

Symbol	**Meaning**
%	Percent
°C	Degree Centigrade
µm	*Micrometer*

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Chapter One

Introduction and Basic Concept

CHAPTER ONE

Introduction and Basic Concept

1.1 Introduction

Tooth enamel is a protective layer that surrounds the tooth's crown and could be seen with the human eye. Enamel is the most mineralized tissue in the body, with 96 % inorganic substance, 4 %organic material and water, a very hard substance that acts as a barrier between the tooth and the environment, although acids from food and drink can destroy it (Mount et al., 2016, Ross and Pawlina, 2006).

Tooth decay is an infectious microbiological disease that affects people all over the world. According to the World Health Organization (WHO), dental caries affects 60 to 90 % of school-aged children(Elfagi, 2021). Dental caries is a complex illness that causes demineralization of tooth structure in both children and adults. Endogenous cariogenic bacteria, regular consumption of fermentable carbohydrates, and a susceptible tooth and host are the key causal reasons Although untreated dental caries can progress, it can be prevented, reversed, or arrested in its early stages (Sharma and Puranik, 2015). When a lesion is discovered early enough, it can be treated, dental caries is addressed using a novel concept of prevention and minimal intervention treatment. Antibacterial agent chlorhexidine has been shown to prevent dental cavities. Diet and fluoride have also been shown to aid in the prevention and treatment of tooth decay (Gugnani and Gugnani, 2017).

Fluorides were substantially responsible for the decline in dental cavities in developed countries during the last few decades. Dental caries is the most common non-communicable disease in the world. The surface-based susceptibility hierarchy indicates that surfaces in the same group have similar susceptibility to caries, where the most susceptible group consists of occlusal and buccal surfaces of lower first molars and the least susceptible surfaces are smooth and proximal surfaces of first premolars, canines and incisors (Stangvaltaite-Mouhat et al., 2021).

Fluoride was thought to be most effective in preventing new incipient and advanced caries on occlusal surfaces, but not on approximal or smooth surface caries. Fewer occlusal and smooth surfaces with incipient caries progressed to advanced caries (Tadakamadla et al., 2020). Fluorides in their systemic form are the ones that can be consumed. Fluoride that is taken systemically combines with the tooth components and structure during the creation of the teeth prior to eruption. It can convert hydroxyapatite to fluorapatite, making teeth more caries resistant (Harris and Garcia-Godoy, 2004).

Fluorides used topically can help with remineralization. Fluoride particles in saliva are increased by local fluorides, leading fluorapatite to develop more quickly. Acid attack and demineralization are not a problem for fluorapatite. Furthermore, increasing fluoride concentrations has been shown to limit bacterial metabolism (Rošin-Grget et al., 2013).

After decades of development, laser technology has reached a high level of refinement, and more advancements are possible. Additional applications for laser-based photochemical processes are being explored, including the ability to target specific cells, diseases, or chemicals. Specific laser technologies are projected to become significant components of contemporary dentistry practice (Pendyala et al., 2017).

In 1997, the Er.Cr:YSGG laser were introduced for surgical needs. Er.Cr:YSGG pulsed laser has been proposed as a candidate for successful tissue ablation, melting and recrystallization because of its strong absorption coefficient, which allows for substantial power deposition with low thermal propagation. This reduces thermal causalities while increasing ablation frequencies. The Er.Cr:YSGG laser works at 2780 nm in the infrared spectrum and ensures the maximum level of hydroxyapatite (HA) plus water absorption spectrum. Since laser irradiation, the calcium-to-phosphorus ratio, carbonate-to-phosphate ratio, and inorganic and organic element composition of hard tissues change, resulting in modification the compounds and response to acid attack and decay susceptibility, and suggesting that laser etching may cause re-mineralization. microspaces that trap free ions, reducing acid damage and caries sensitivity (Erkmen Almaz et al., 2021a).

1.2 Dental Tissue

Enamel, dentine, pulp, and cementum make up the tooth structure. Dentin, which makes the majority of the tooth structure, is an inert, hard, acellular tissue created by epithelial cells and reinforced by less mineralized, more resilient, and vital hard connective tissue (70% of the dentin is made up of hydroxyapatite crystals, 20% of the dentin is made up of organic material including fibrous collagen, and 10% is water). The mineralized dentin and pulp tissue that are responsible for the construction and maintenance of the tooth bulk are referred to as the "dentin-pulp complex." The root canal is a channel that allows nerves and blood vessels to access the pulp cavity through the root of the tooth. An extra layer of bony material, cementum, ordinarily surrounds the root as shown in Figure (1.1) (Nanci, 2017).

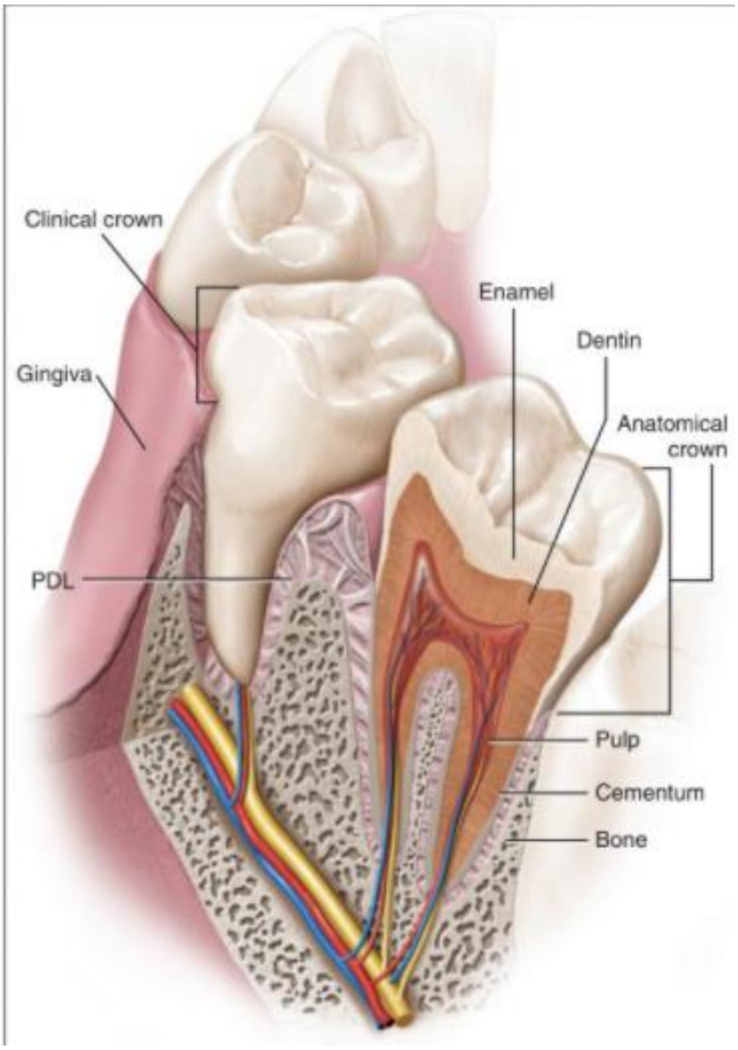

Figure (1.1) ; Tooth Structure (Nanci, 2017)

1.2.1 Dental Enamel

It acts as a protective cover for the tooth, with varying thicknesses covering the entire crown surface. Enamel thickness on the cusps of human teeth ranges from 2 to 2.5 mm, with the crown neck being the thinnest. Enamel forms a protective layer around the teeth, allowing them to serve mastication.

The color of the enamel that covers the crown varies from yellow white to grayish white. Because of the character of enamel, which is thin transparent that covers the yellow dentin, they can look yellowish in color. It is color may also be attributed to differences in enamel homogeneity and degree of calcification. Where the thin edge consists only of a double layer of enamel, the incisal edge may have a bluish shade (Xuelian et al., 2016). Enamel is a highly mineralized crystalline structure that contains 96% inorganic materials by weight, 4% organic materials by weight, and water. The backbone of enamel is hydroxyapatite crystals which is primarily calcium and phosphate. The mineral portion of the enamel consists mainly of carbonate ions (about 2-5 percent w/w) as well as a small percentage of trace elements such as Mg,Cl, K, Sn, Sr, Fe, Ni, Cr, Co. As a result, the mineral portion of enamel is best known as carbonated HA. Enamel carbonated HA is classified into two forms based on the placement of the carbonate ion within the crystal lattice: type A and type B. When carbonate ion replaces the hydroxyl (OH-) within the crystals, Type A carbonated HA is formed. According to study, Type A carbonated HA accounts for just 11% of total carbonated HA in tooth enamel composition. Type B carbonated HA (89 percent of overall enamel carbonated HA) is generated when the carbonate ion replaces the phosphate group (PO_4-3) inside the crystal (Smith, 2020, Gil-Bona and Bidlack, 2020, Yamakoshi et al., 2006). During tooth enamel growth, ameloblast cells secrete the organic matrix of the tooth enamel, which accounts for about 20% of the content of human fetal enamel. However, in mature enamel, this percentage drops rapidly until it reaches 2-4 %. Ameloblast cells produce enamel, which is damaged as the tooth erupts into the oral cavity and cannot be replaced. The period of matrix development, the secretory stage, and the period of maturation, which consists of the continued growth of mineral crystals that develop instantly after starting matrix production, as well as the loss of protein and water, are the three major stages of amelogenesis (Mescher, 2013). The basic unit of enamel, the enamel prism (enamel rod), is a tightly packed mass of hydroxyapatite crystals in an ordered shape, typically measuring 4–8 μm in diameter. During mastication, prism is thought to act as structural reinforcement (Mescher, 2013, Lucas et al., 2008).

1.3 Dental Caries

Dentistry extends back to 5000 BC, when a "dental worms" was supposed to be the source of dental cavities. The term dental caries first appeared in print in 1634, and it is derived from the Latin phrase caries, which meaning decay. The term "tooth cavities" was defined as defects in the teeth. According to specialists, dental caries is one of the most prevalent and ancient illnesses in humans. Dental caries is a highly infectious illness caused by dental microbial species such as "Streptococcus mutans", which demineralizes natural teeth and causes tooth decay over time by breaking down carbohydrates due to acid generation (Rathee and Sapra, 2020).

Dental caries seems to be a complicated condition that is "simple in theory but complex in practice"(Pitts et al., 2017).

The basic conditions for caries to develop are as follows (Rathee and Sapra, 2020):

- Plaque bacteria.
- A suitable tooth surface.
- Dietary fermentable carbohydrate (e.g. sugars)
- Time.

However, if these factors are not under the control of the person, it is affected by other variables such as biology, socio-economic, cultural, environmental conditions, and lifestyle. It's important to remember that any preventive strategy must address both the underlying determinants and the immediate causes of illness (Freedman, 2011).

Dental caries has been recorded as the most frequent chronic childhood condition, and it still affects a significant number of people in some areas. Increased sugar intake and insufficient fluoride supply are the main causes of this excessive manifestation in some persons (Freedman, 2011).

The physicochemical conditions of mineral dissolution are needed for the onset of dental caries. Caries is a progressive phase of repeated acidic conditions below essential pH (5.5), where the balance between remineralization and demineralization tipped towards demineralization with the production of clinically detectable white spot lesions (Freedman, 2011).

A saliva-modified bacterial infection *(Streptococcus mutans*) and dietary carbohydrate cause an imbalance between de-mineralization and re-mineralization of the tooth enamel matrix, resulting in white spot lesions (Khoroushi and Kachuie, 2017). The existence of a white spot lesion, a patch of opacity, provides clinical evidence of the lesion initiation. At this point, the carious process can be paused or reversed (Sadikoglu, 2020). So, depending on the oral cavity system, which may causes bacterial plaque to build up and stay on the enamel tooth surface over time, as well as the individual's oral hygiene and inherent resistance, caries can be initiated and prognosed (Sadikoglu, 2020). simply the accumulated plaque of bacteria, time of demineralization exceeds the rate of remineralization over a long period of time (more than half hour) produce lesion (Rathee and Sapra, 2020).

1.3.1 Risk factors

The majority of caries risk factors/indicators fall into one of the categories below (Freedman, 2011) :

1.3.1.1 Social history

In children under the age of 12 years, evidence for an inverse association between dental caries and socioeconomic position was very strong, although there was less evidence for the same detrimental connection in older children (Freedman, 2011).

1.3.1.2 Medical conditions

Medical disorders, can put a patient at a high risk of acquiring dental caries, for example:

- Disorders that impair a patient's ability to handle their entire oral health, such as mental or physical disabilities, may have an effect on oral health. (Berkey and Scannapieco, 2013).

- Medical conditions may cause xerostomia , whether a side effect of treatment or as a result of the disease itself, may place a patient at high risk for dental caries (Han et al., 2015, Devalia and Hood, 2021).
- Caries development and/or treatment can put a patient's general health in risk in several medical circumstances (e.g. , immunosuppression and bleeding disorders such as haemophilia (Devalia and Hood, 2021).

1.3.1.3 Dietary habits

At a time when fluoride exposure is common, a systematic review looked at the connection between caries and sugar consumption. The researchers hypothesized that fermentable sugars and carbohydrates are important in the incidence and development of caries. and they sought to explore how fluoride influenced the sugar-caries association. As a result, the connection between caries and sugar intake has weakened in the modern era of fluoride exposure, but it should be noted that limiting sugar consumption is a reasonable part of dental caries (Rizzardi et al., 2021).

It was discovered that the balance between "bad" and "good" It is necessary to safeguard children under the age of six from dental caries risk factors (i.e. extremely cariogenic diet) and "normal" (i.e. tooth-brushing) The importance of good dental hygiene habits in the prevention of dental caries cannot be overstated (Rizzardi et al., 2021).

A number of diet-related to dental caries risk, as (Parnell et al., 2002):

1. after 2 years of age, ablactation from the infant bottle.

2. Drinking or eating sweet snacks in between meals.

3. Taking a baby bottle to bed in sleeping.

4. Drinking juice from a baby bottle.

Rather than invasive restorative therapy, modern dentistry focuses on primary prevention of dental caries. Despite substantial strategies to encourage fluoridation and oral cleanliness, public health and dental science continue to face obstacles in preventing and treating early dental caries lesions, particularly for those who are at a risk of developing caries, the most commonly encountered illness (Hannig and Hannig, 2019).

1.4 Mineralization processes of the enamel

During the day, the enamel of a tooth is subjected to several demineralizations –remineralizations processes. The balances between remineralizations and demineralizations processes determine whether dental caries will remain stable, advance, or reverse. (Siddiqui and Saba, 2020). Biomineralization is a continuous, complex, and dynamic process. Acids from foods and drinks, as well as bacterial assault, promote demineralization of teeth (Gewargis, 2017). These acids cause pH to drop, causing chemical breakdown of both organic and inorganic matrixes, as well as enamel mineral loss. Enamel demineralization occurs when phosphate & calcium ions seep into the mouth. (Siddiqui and Saba, 2020). If the lesion is diagnosed before cavitation and the quantity of cariogenic bacteria is lowered, this can be reversed and stopped. Calcium & phosphate ions travel first from tooth to the mouth throughout demineralization, and vice versa at remineralization. Fluor apatite is a mineral that can withstand demineralization while simultaneously promoting remineralization (Gewargis, 2017). Treatments for remineralization, using synthetic hydroxyapatite, can also help to prevent demineralization and accelerate remineralization. Synthetic hydroxyapatite can attach to tooth surfaces and clog dentin tubules, forcing them to remineralize (Pałka et al., 2020). Patient education, free sugar restriction, plaque control, saliva buffering capacity, salivary flow rate, and additional caries-prevention treatments like as antibacterial agents and fluoride can all help to break the cycle (Uma Maheswari, 2007)

1.5 Prevention methods

1.5.1 Pit and fissure sealants are used.

Dental sealants were created in the 1960s to decrease and minimize occlusal cavities pits and fissures. since that, use of such sealants increased. Sealant acts as a physical barrier, preventing food and germs from accumulating in deeper fissures and causing caries. Isolation and etching are required before applying sealing chemicals. The use of a sealer helped to decrease caries. After a year, the caries rates of sealed and unsealed teeth were compared, and the sealed group had an 87 % lower caries rate. Furthermore, 4–4.5 years post sealant therapy, a 60 % contribution in caries was seen (Ahovuo-Saloranta et al., 2017)**.** On the other hand, the probability of sealant success was greatly reliant on the therapeutic strategy and application. The patient's motivation to engage in the etch and dry process is crucial, and it can have a negative impact. It can shorten the life of the sealant if done incorrectly (Chestnutt et al., 2012).

1.5.2 Xylitol

Xylitol is an anti-caking sugar that can be found in chewing gums, oral syrups, and candies. Organic acid byproducts are not deposited in biofilm because cariogenic bacteria cannot metabolize this sugar. By boosting saliva output, xylitol chewing gums also help to prevent cavities (Nayak et al., 2014).

1.5.3 Dentifrices and mouthrinses

Dentifrices and mouthrinses containing various calcium compounds are available. Calcium compounds that are saturated, such as amorphous calcium phosphate (ACP). The use of nanohydroxyapatite (nHAP), tricalcium phosphate (TCP), and calcium sodium phosphosilicate (NovaMin) in the prevention of tooth demineralization and improvement of remineralization has been described. (Patil et al., 2013).

1.5.4 fluoride

Fluoride plays a vital function in tooth structural resistance to demineralization. In developed countries, fluoride ions (fig. 1.2) have been found to lessen the occurrence and severity of dental cavities. Fluoride, which is accessible in 0.7 mg/L in public water supply, is one of most cost-effective treatments of caries control. Fluoride in tiny amounts is beneficial for caries prevention, but excessive amounts cause fluorosis, which produces enamel discoloration and defects known as mottled enamel (Pajor et al., 2019).

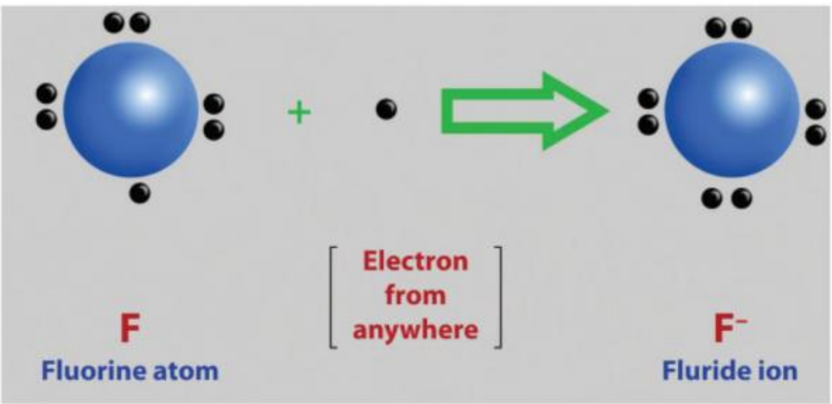

Figure (1.2): atomic structure of fluoride ion (Pajor et al., 2019)

Bacterial acids degrade enamel by lowering the pH of saliva. Calcium and phosphate ions dissolved on the enamel surface and flow into the mouth. Salivary flow, protein, calcium, and phosphate levels, as well as fluoride levels in plaque or saliva, can all aid to restore mineral loss. The transport of phosphate & calcium ions out of and into the enamel distinguishes the dynamics of remineralization and demineralization (Featherstone, 1999). Ion exchange between hydroxyapatite and the fluoride system is thought to occur when a fluoride system with low levels (like toothpastes and mouth rinses) is applied topically. When a small amount of fluoride is mixed with hydroxyapatite, a fluoride ions in the crystal replacing the hydroxyl ions, forming fluorapatite (figure 1.3) (Rošin-Grget and Linčir, 2001, Pajor et al., 2019).

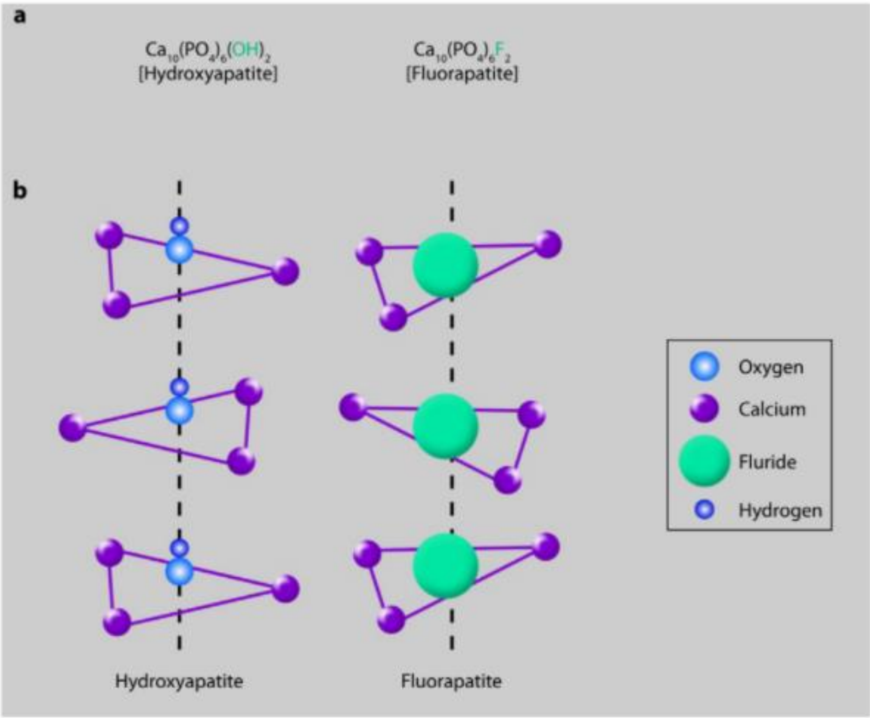

Figure (1.3): Exchange of fluoride ion with hydroxyl groups in hydroxyapatite structure and formation fluoroapatite (Brugnera and Namour, 2018)

Crystals of fluorapatite are more stable than those of hydroxyapatite. Even in the case of severe enamel fluorosis, full substitution of hydroxyl groups is hardly achieved (Pajor et al., 2019). On the enamel surface, fluoride can only replace 10 % of hydroxyl group. The hydroxyl sites are partially replaced by fluoride ions, resulting in the production of the hydroxyapatite–fluorapatite complex. This flurohydroxyapatite crystal is more abundant. It is more resistant to acids and has a lower overall solubility (Rošin-Grget and Linčir, 2001). Fluorapatite crystal formation can be a mechanism of fluoride response when the saliva is supersaturated with fluoride, in addition to ion exchange of fluoride and hydroxyl groups following long-term exposure to low levels of fluoride through systems such as dentifrices and mouth rinses (Štepec and Ponikvar-Svet, 2019) .

Calcium fluoride formation is the third mechanism of fluoride reaction with apatite, and it is the only consequence that happens on enamel during short fluoride exposure to high fluoride concentration agents like NaF or APF (Fulaih, 2018, Rošin-Grget et al., 2013). Fluoride, on the other hand, can be adsorbed onto hydroxyapatite crystals in enamel. Alkali-soluble calcium fluoride and adsorbed fluoride (Rošin-Grget et al., 2013).

These alkali-soluble fluorides are also known as calcium fluoride and loosely bound fluoride. When the tooth is exposed to acid attacks, the calcium fluoride bonded to the enamel surface functions as a reservoir to release fluoride to the saliva, and plaque is progressively released later. By a variety of methods, fluoride slows demineralization of the tooth structure and reduces the tooth's susceptibility to caries. The ions dissolve in hydroxyapatite and fill or replace the hydroxyl ion gaps in the crystal, stabilizing and lowering the crystal structure's solubility (Fulaih, 2018).

Despite the fact that all of the above preventive techniques have been shown to be successful in the literature, restorative treatments are still required. All of the above preventive interventions, with the exception of water supply fluoridation, are reliant on patient compliance and regular and frequent recall procedures. Patients may not be sufficiently encouraged to take care of their oral health due to systemic limitations or economic challenges in nations lacking related government support and health insurance systems. Furthermore, when people age and develop gingival recession and root caries, they are unable to attend regular dental checkups for caries prevention (Brugnera and Namour, 2018).

They are also more likely to acquire caries as a result of the medicines they take, which might impair salivary gland function. Adults who wear orthodontic or removable partial appliances, or who have orthodontic brackets on their teeth, are more likely to develop dental cavities as a result of the difficulties they face in keeping their teeth clean. Fluoride application and types or methods are summarized in table (1.1) (Brugnera and Namour, 2018).

Table (1.1): Fluoride treatment options (Brugnera and Namour, 2018).

Application Method	**Fluoride Compound**	**Application**
Systemic application	Public water supply (0.7 ppm)	The most cost-effective method; best for children with developing permanent teeth
	Dietary supplements (tablets, drops or lozenges)	6 months to 16 years children in nonfluoridated areas and at high risk for caries
Professional application	NaF 5% varnishes	High risk patients; especially children in high risk for caries
	APF 1.23% Gels	
	TiF4 1%	
	SnF 8% solutions	
Patient application	NaF 0.05% & 0.2 % mouthrinses	High risk patients; as an additive to systemic or professionally applied fluoride
	Fluoridated dentifrices	All patients

1.5.4.1 Acidulated phosphate fluoride (APF)

APF gels contain 12 300 ppm fluoride which are made up of sodium fluoride, hydrofluoric acid, and orthophosphoric acid. There are also monobasic sodium fluoride, phosphoric acid, and sodium phosphate gels with a concentration of "5000 ppm F" (Raghavan et al., 2022).

• Fluoride gels with such high concentrations of fluoride should only be used by professionals and should not be sold to the general public.

• Thixotropic gel flow under pressure in the trays, allowing the gel to enter between the teeth (Cameron and Widmer, 2013) .

Acidulated phosphate fluoride, was developed in the 1960s (APF). This approach was created by Brudevold et al (Newbrun, 2011). These scientists studied fluoride's chemical interactions with enamel (hydroxyapatite) and arrived to the following conclusions:

(1) To increase the rate of fluoride reaction with hydroxyapatite, the pH of the fluoride system could be increased acidic (AL-Hasnawi and Al-Obaidi, 2014).

(2) If phosphoric acid was employed as the acidulant, the concentration of phosphate at the reaction site would be increased (AL-Hasnawi and Al-Obaidi, 2014).

The anti-caries activity of topical fluoride is influenced by the reaction compounds generated on the enamel after therapy and their long-term preservation. The fluoride content and pH of the commercial entity utilized have an impact on this procedure (Raghavan et al., 2021).

In a study of enamel rigidity to a cariogenic threat after usage of an APF or NaF conditioner, greater fluoride absorption and a more effective decrease in demineralization were observed in APF-treated enamel compared to NaF-treated enamel (Delbem and Cury, 2002). Using acidulated fluoride gels with one as well as four minutes has a same effect on enamel susceptibility to carious lesions formation, according to some studies (Delbem and Cury, 2002, Villena et al., 2009). APF was created based on research that aimed to speed up the synthesis of enamel fluorapatite, reduce calcium fluoride formation, avoid demineralization, and boost fluoride absorption at the surface (Limeback and Robinson, 2012). Previous research has indicated that lowering the pH of a solution to 4.0 enhances fluoride absorption into the enamel; nevertheless, acidic solutions like hydrochloric or acetic acid cause enamel decalcification. Phosphoric acid was chosen as the acidulate because it did not cause demineralization (Hassanali, 2018).

From an evidence-based standpoint, the effectiveness of topically applied fluoride products in the prevention of caries in children and adolescents is well established, although regular use of high-frequency, low-concentration fluoride regimens for caries prevention and control is recommended regardless of the patient's caries risk or behavior, the high-concentration, low frequency fluoride application with APF or NaF gel is required in situations of high risk or carious activity (Poulsen, 2009, Sobral et al., 2009).

The high fluoride concentration found in the outermost enamel, according to the scientists, is acquired during pre-eruptive maturation, when the enamel is more porous. Increased fluoride concentrations were not observed in subsurface fissure enamel lesions, meaning that fluoride does not diffuse to the interior of the deepest part of the lesion and therefore has little effect on its progression (O Mullane et al., 2016).

In a range of health-care areas, including dentistry, lasers are currently the subject of substantial research and public interest. Caries-prevention lasers are advertised on several lasers. The use of lasers to prevent caries reduces the need for multiple visits to the dentist for fluoride treatments(Brugnera and Namour, 2018)

1.6 Laser basics

The term Laser is an abbreviation of "Light Amplification by Stimulated Emission of-Radiation". Laser beam is an electromagnetic radiation that are related to ultraviolet, visible and infrared regains of the electromagnetic spectrum. (Mahamood, 2018), as seen in the figure (1.4):

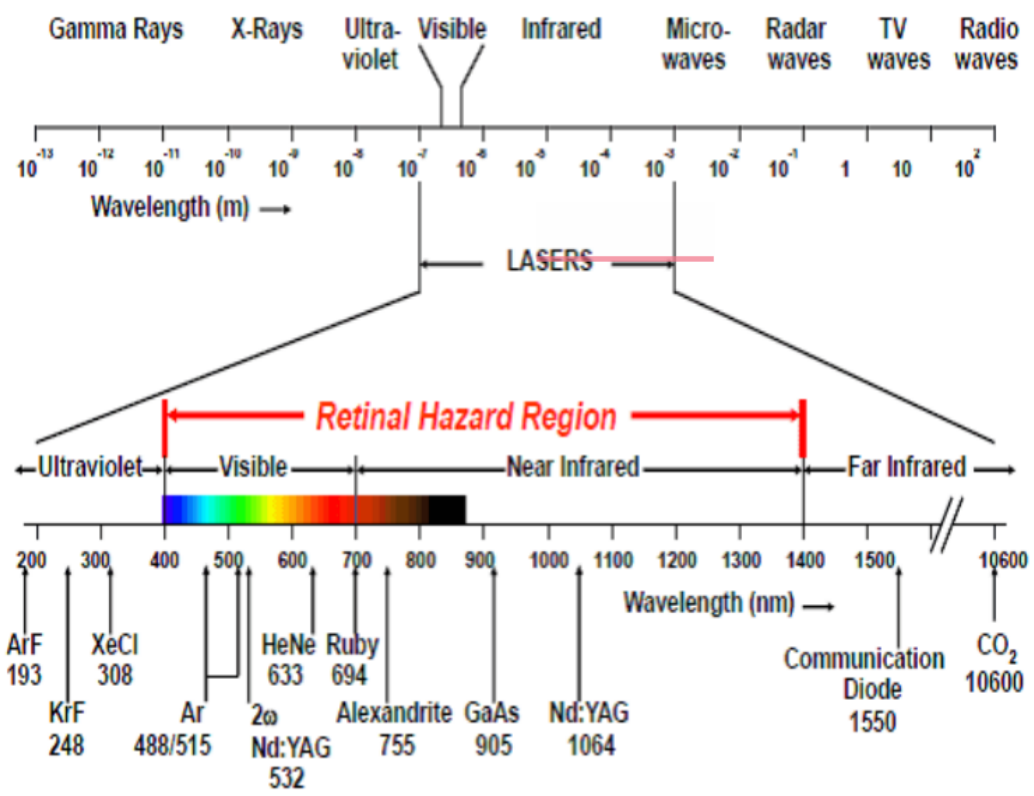

Figure (1.4) : Laser wavelengths in electromagnetic spectrum.(Brugnera and Namour, 2018)

1.6.1 History of the laser

In 1916 Albert Einstein suggested that under optimum conditions atoms may release super flowing energy as light either spontaneously or when stimulated by light. The stimulated emission first observed by Rudolf Ladenburg in 1928. In 1953, Charles Townes announced his new innovation " MASER" which stands for microwave amplification by stimulated emission of radiation, he excited and amplified ammonia particles through resonant microwave cavity so they emitted microwave wavelengths.

Later on, many researchs done on maser by Townes and Schawlow to expand its emission to shorter wavelengths (infrared and visible light) followed by Gordon Gould studies which yields the new innovation "Laser". Theodore Maiman used a camera flash to excite chromium in ruby crystal then red pulses from the ruby crystal was emitted. In the late of 1960 the first gas laser (He-Ne laser) produced with a continuous IR beam emission. In the next year the first semiconductor laser invented at general electric company by Robert Hall and his colleagues. In 1964 CO_2 and Nd:YAG lasers were constructed (Parker, 2007, Hecht, 2010).

1.6.2 Components of laser system

The main components of laser system are shown in figure (1.5), it described as follow:

A) active medium:

It is the material within laser cavity that absorb the pumped energy and emits coherent light as a result of atomic or molecular transition from the higher energy states (to which they were previously stimulated) to the lower energy states. There are solid, liquid and gas active media (Aleshin et al., 2019).

B) Reflecting mirrors:

Two main mirrors or polished surfaces are located at the laser cavity's two ends (in case of semiconductor laser). The reflectivity of one mirror is 100% while the other is partially reflecting mirror (10%-99%). These mirrors function as optical mirrors by reflect that radiation backward and forward through the active medium helping in amplifying and collimating the resulting beam by making the photons circulating many times through the laser cavity. The directionality of the emitted beam controlled by these two mirrors. (Convissar, 2015)

C) **Pumping mechanism**:

It is an excitation source that supply the atomic system of the active media with an amount of energy to make transition from lower to higher energy states (population inversion). There is optical pumping system such as flash lamp, electrical pump system (electrical discharge through gas medium) and chemical pumping.(Convissar, 2015, Taylor, 2017, Pavel et al., 2014).

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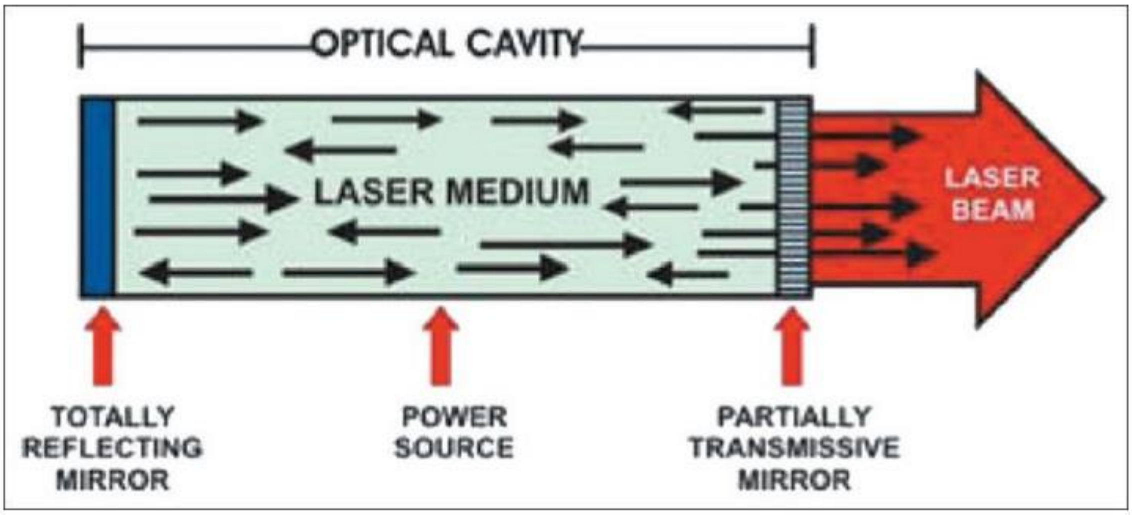


Figure (1.5) : Major parts of laser unit.(Demtröder, 2013)

1.6.3 Characteristic features of laser radiation

A) **Monochromaticity:**

It means single wavelength. In fact laser emits a narrow band of wavelengths so it is closer to the monochromaticity than any other ordinary light (Kaminskii, 2013) .

B) **Directionality:**

The emitted laser beam is nearly straight line and if traveled for long distance it will deviate from its straight path due to diffraction (Kaminskii, 2013, Al-Aamirry, 2003).

D) Coherence:

Ordinary light is not coherent as it originates from various atoms. As a consequence of the stimulated emission, coherence develops (making it a unique property for laser light) by which the emitted photons are all in phase in relation to each other both spatially and temporally (Flanders et al., 2019).

E) Collimation:

Laser beam is composed of a number of waves that move in the same phase and parallel to each other with very small divergence between them. In contrast to conventional light, which lacks this feature, the collimation property of lasers allows a laser beam to be concentrated to a very tiny region with high intensity (Roy et al., 2015).

1.6.4 Mode of Operation

As time passes, dental laser equipment could generate light energy in two modes (Pendyala et al., 2017):

1- constant.

2- Pulsed.

The target tissue receives energy from pulsed lasers, which are divided into gate and free-running modes. Three unique emission modes have been found as a result (Coluzzi and Parker, 2017, Pendyala et al., 2017):

1. The beam is supplied at a consistent power level for the duration of the foot switch being pressed in continuous-wave mode.

2. Gated-pulse mode, the laser energy alternates on a regular basis, It's the same as a flashing light. A mechanical shutter is open and close in front of the continuous-wave emission's beam direction to achieve this mode. This gated pulse function is included in most surgical equipment that operates in continuous wave mode.
3. Pulsations in a free-running mode True pulsed mode is how this mode is described. High peak intensities of laser beam are generated about microseconds before the laser is turned off for an extended period of time, making this form of emission unique**.**

1.6.5 Laser effects on biological tissue

When laser irradiate tissue there are four effects may appear (Parker, 2017), figure (1.6):

1. Absorption: as laser beam hits tissue, the medium (tissue) will attenuate laser's energy transforming it into another form relying on amount of energy in incident beam (either heat or biostimulation)

2. Reflection: Total reflection of an incident beam happens when the incidence angle is smaller than that of the refraction angle. The angle of incidence and reflection will be the same in perfect reflection. Scatter is a possibility if the interface is inhomogeneous or rough.

3. Transmission: laser beam does not undergo any interaction with the medium during its passage through it, then it will emerge out unchanged or partially refracted.

4. Scattering: It happens when the laser beam interacts poorly with the tissue, resulting in insufficient attenuation of the incident beam. The beam rays move in uncontrolled directions and its energy will decreased with distance and some distortion will occur. When laser beam of short wavelength hits the tissue back scattering may result.

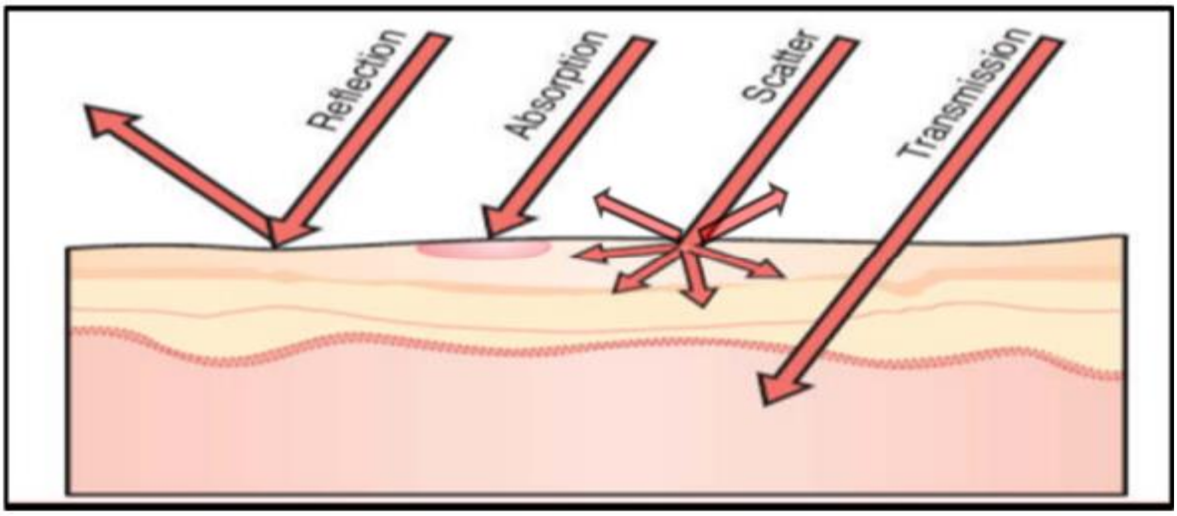


Figure (1.6): Laser effects on tissue (Pendyala et al., 2017).

1.6.6 Laser tissue interaction

The different medical applications of laser relies on the ability to induce local necrosis, etching, or fragmentation of target tissue which determined on the basis of tissue characteristics and laser beam. Various interaction mechanisms result when laser energy hits biological tissue, these are categorized as follow (Niemz et al., 2004):

1.6.6.1 Wavelength dependent mechanism

I. Photochemical Interaction

An interaction between laser beam and macromolecules in target tissue could results in photochemical effect and reactions. The mechanisms of photochemical interaction are biostimulation and photodynamic therapy (PDT). Photochemical interactions occur at lower energy densities and over longer time durations, ranging from seconds to continuous exposure (Mahamood, 2018). Photochemical reactions used in curing light cured composite resin fillings and in periodontal pocket and root canal disinfection. The disinfection achieved by adding photosensitive agent to the area to be treated then by applying laser power on these photosensitizers the chemical bonds (of photosensitizer) will be braked leading to a release of singlet oxygen radicals which have strong bactericidal effects. (Convissar, 2015)

1. photodynamic therapy (PDT)

As mentioned above PDT based on photochemical reaction and need the presence of three elements: laser power, photosensitizer, and molecular oxygen (Brugnera and Namour, 2018). The procedure starts by injecting photosensitizing agent (e.g. porphyrin) into the body then waiting for period of hours in order to ensure the release of photosensitizer from the normal tissues. Then using laser beam to excite this agent. The sensitizer after stimulation will undergo series of intramolecular chemical reactions that lead to release of reactive oxygen radicals which in turn result in oxidation of cellular components (i.e. cytotoxicity). As the photosensitizer persists for longer time in tumor tissue than in healthy tissue, selective destruction for tumor tissue will occur while healthy tissue stays safe (Knappe et al., 2004).

2. Biostimulation

Low level laser therapy (LLLT) is a type of therapy that uses only one wavelength of a light to activate or inhibit a biological process. LLLT doesn't elevate tissue temperature, instead it produce the effect though photobiostimulation within the target tissue (Suresh et al., 2015). The laser dose implied in LLLT lies within the range between 0.001- 10J/cm^2. The photonic energy absorbed by cells affect cellular metabolism and multiple signaling pathways. By which the mitochondria will increase adenosine triphosphate (ATP) production which in turn facilitates oxidative and other cellular process and result in enhancing healing, vascularization, growth along with other mechanisms (Peševska et al., 2006).

Elson and Foran studied LLLT effects in the oral cavity and they found that it is an efficient and safe method in healing enhancing, pain relief, and it decrease inflammation in the mouth along with the advantage that it reduce the need to post-operative medicament (Al-Farawn et al., 2019) .

II. Thermal interactions

Photothermal interactions achieved when laser energy transformed into heat inside tissue. This is typically occurring at power densities between 1-106 W/cm^2 with exposure time ranging between 1µs-1min. As tissue temperature elevated, many changes will appear. The first to appear is hyperthermia which is elevation in tissue temperature above 37°C up to 50°C and the last thing to occur is tissue charring at temperature exceeding 200 °C, (Knappe et al., 2004). figure (1.7) illustrates the different thermal effects on tissue.

Table (1.2): The photothermal effects on tissue when absorbing laser radiation (Pendyala et al., 2017) .

Temperature	Tissue changes
37°C	There is no obvious effect on tissue at this temperature or even if it exceed 5 °C above it .
42-50°C	The first is thermal effect can be contributed to changes of molecular conformation characterized by membrane alterations and bond destruction. This is called (hyperthermia) which lasts for several minutes and at this time necrosis occur in significant percentage of the tissue cells .
60°C	Elevation of temperature to this level will cause proteins and collagen denaturation which lead to tissue coagulation and cells necrosis; it is obvious macroscopically as visible tissue paling. There are numerous treatment techniques such as LITT require an elevation of temperatures to just above 60°C.
>80°C	Excessive increase in the permeability of the cell membrane which will destroy the maintained balance of chemical concentrations within cells.
100°C	Vaporization of water contained within tissues cause large increase in volume and gas bubbles formation which induce mechanical ruptures and thermal decomposition of tissue fragments
>150°C	This appear as blackening of the nearby tissue, smoking and charring (Carbonization) .
>300 °C	The tooth substance mostly consists of hydroxyapatite crystals (the chemical compound of calcium and phosphate ions) which undergo melting when the tissue temperature elevated to few hundred degrees Celsius.

III. Photoablation

Photoablation occur when the photonic energy is very high which is available in UV lasers (e.g. excimer laser). This is typically achieved with power density threshold at 10^7 -10^8 W/cm^2 and with pulse duration within nanoseconds. At these parameters laser beam does ablate tissue efficiently without any thermal damage to the neighboring tissues. Photoablation widely used in refractive corneal surgery (Steiner, 2011, Convissar, 2020).

1.6.6.2 Wavelength Independent Mechanism

I. Plasma induced ablation

When laser applied at very high-power densities over 10^{11} W/cm^2 in solid and liquid materials and 10^{14} W/cm^2 in gases, optical breakdowns result. When the electrical fields are intense enough to take off electrons from the atoms this will results in ionization in the medium and plasma formation. Tissue removal by plasma induced ablation is recognized to be extremely clean and precise, with no mechanical or thermal damage (Steiner, 2011, Tsai et al., 2009).

II. Photodisruption

In photodisruption three things occur; optical breakdown, plasma formation, and generation of shock waves. This could result in formation of cavitation and jet formation when the optical breakdown take place within fluid or soft tissue, then the tissue will be fragmented by mechanical force. Photodisruption considered a mechanical effect created by ultrashort laser pulses within picoseconds and femtoseconds which in turn leading to the creation of pulses that have very high peak powers. The medical usage of this mechanism essentially in refractive corneal surgery by using titanium sapphire laser with pulse duration about 100 fs, also used in removal of hard and soft tissue because its cutting is considered precise but not so efficient (Steiner, 2011, Lin et al., 2010).

1.6.7 Laser safety

Laser safety measures such as protective goggles and flammability are ruled by laser classification which made by the American National Standard Institute (ANSI) which classified lasers according to their powers and their possible injuries to the operators (Wamsley et al., 2021):

• **Class 1**: lasers are safe to use under normal circumstances as they don't cause skin or eye damage.

• **Class 1M**: these lasers are considered safe during exposure when viewed without collecting optics (e.g. loups, microscope) and with these collceting optics it may cause damage.

• **Class 2:** If viewed in less than a quarter second (0.25s), lasers of visible wavelengths (400-700 nm) do not cause skin or eye damage.

• **Class 2M**: lasers with visible wavelengths that have potential hazard during exposure with collecting optics.

• **Class 3R**: lasers within this category causes serious injury to eyes if they focused on direct laser beam or even reflected beam with no apparent injury to the skin.

• **Class 3B**: include visible and invisible lasers with medium powers, these lasers could cause potential damage to the eyes by their direct and reflected beams and in case of high power lasers, scatted beam could results in skin injuries.

• **Class 4**: include lasers in visible and invisible spectrum region they can cause injuries to the skin and eye even when not directed to them (the scattered beam could result in potential injury), another thing these lasers could cause fire hazard and byproduct emission hazard.

1.6.8 Types of some lasers

1.6.8.1 carbon dioxide Laser

In Carbon dioxide laser, the lasing or active medium is carbon dioxide (in addition to other gases needed for sustained stimulated emission of radiation). The gases are either contained in a tube or released from a storage tank. (Witteman, 2013, Katzir, 2012). CO_2 lasers used to emit energy in a continuous wave which could be gated with later upgrades, but only at greater mJ and prolonged pulses heat damage to surrounding tissue increased as a result of the less advanced technology of those units, resulting in charring (Aoki et al., 2004).

10,600 nm CO_2 lasers used in the most up-to-date technology for producing CO_2 laser energy. Treatment is made easier by using an articulated arm in a noncontact mode. This wavelength has a penetration depth of 0.5 mm and interacts with water and hydroxyapatite. Inflamed tissue, like crevicular fluids and intracellular fluids, has a higher water content and is thus influenced by laser energy. Photothermally heated fluids are vaporized, causing cell membranes to collapse. When energy is applied, the bacteria are inactivated, and dehydration occurs (Crespi et al., 2005, Kojima et al., 2005). Despite the fact that CO_2 laser irradiation has been shown to reduce enamel demineralization, it is better for dental enamel because it emits infrared light 9300, 9600, 10300, and 10600 nm that nearly resembles various apatite absorptions, particularly that of the phosphate and carbonate group (Luk et al., 2020). As a result, better caries prevention efficacy with less deleterious effects on dental tissues could be attained (Caneppele et al., 2020, Khamverdi et al., 2018). This laser takes energy from the exterior enamel surface for a few micrometers and transforms it to heat, causing mineral carbonate loss and hydroxyapatite crystal fusion, reducing interprismatic gaps. Furthermore, it boosts acid resistance while lowering mineral reactivity and preventing cavities (Khamverdi et al., 2018, Sharma and Puranik, 2015).

1.6.8.2 Erbium Family of Lasers

The FDA has given its approval to erbium lasers for use on both hard and soft tissue. The Er.Cr:YSGG and Er:YAG lasers are free-running pulsed lasers with maximum water absorption, strong hydroxyapatite absorption, and weak hemoglobin absorption. The laser energy has the ability to penetrate up to a 5 µm depth. The high temperature in the surface layers of tissue is low when water is sprayed alongside lasing. Within the pocket, this prevents hemostasis & coagulation. While some people believe that bleeding is a downside of employing those lasers, the benefit of that wavelength is that there is virtually no operating or postoperative pain, as well as speedier healing (Gahlot and Hiremath, 2021). The Er.Cr:YSGG beam has a highly connection again to water and hydroxyl particles that are abundant on the surface of the teeth. As the temperature rises on tooth surfaces, chemical changes in the degree of crystallinity of enamel may increase acid resistance (Ana et al., 2012b, Ana et al., 2006). When this laser is utilized in conjunction with fluoride, the amount of CaF_2-like material deposited on enamel increases (Ana et al., 2012b).

For the prevention of enamel acidity and mineral loss, erbium lasers have indeed been proposed like a non-heated prophylactic technique. The Er:YAG (2940 nm) laser increases the temperature of irradiation enamel while simultaneously altering the chemical and morphological features of the substrate, resulting in a more acid-resistant surface. The Er.Cr:YSGG laser is a dental advanced laser (2780 nm). Despite the fact that it was designed for cavity preparation, research has revealed that the wavelength produced is easily absorbed both water and the hydroxyl radical in hydroxyapatite, implying that it may improve enamel acid resistance (Ulusoy et al., 2020b, da Silva et al., 2019).

When utilized at lower energy densities, In most studies, the Er.Cr:YSGG laser has proved to efficient, is linked to caries prevention and hardness loss reduction on enamel caries lesions, indicating that it could be a better solution for improving enamel acid resistance. Furthermore, these lasers may have a cariostatic effect similar to that of fluoride dentifrice. The chemical examination of postirradiated dental structure with Er.Cr:YSGG lasers revealed an increase in calcium ions and hydroxyapatite crystal rearrangement following laser irradiation, which may be explained by the enamel heating effect.(de Oliveira et al., 2017).

1.6.9 Absorption of laser energy by dental tissues

Water, pigment, blood contents, and minerals are the main oral tissue elements, and the absorption coefficients of various laser wavelengths differ. The main contents are known as primary chromophores, and they are laser energy absorbers. The two erbium wavelengths are most absorbed by water, which is found in all biological tissue, followed by CO_2 (Parker, 2017). On the contrary, shorter-wavelength lasers can be transmitted by water (e.g diode, Nd:YAG). Water and carbonated hydroxyapatite make up tooth enamel. The CO_2 wavelength is readily absorbed by the apatite crystal, while the erbium wavelengths are absorbed to a lesser extent. The shorter wavelengths have no effect on it (Coluzzi, 2008). Since all dental lasers are absorbed by one or more of the soft tissue components, the practitioner should use any available wave length for soft tissue treatments. Erbium lasers with very short pulse durations, on the other hand, effectively ablate layers of calcified tissue with limited thermal effects in hard tissue. Short-wavelength lasers (such as diode and Nd:YAG) are remarkably nonreactive with safe tooth enamel. Using these wavelengths, gingival tissue close to a tooth can be contoured without difficulty. Conversely, if soft tissue impinges on a carious lesion, an erbium laser will effectively eliminate both the lesion and the soft tissue if the proper settings are used for each tissue type. The thickness of a material in which 98 percent of the energy from a laser is absorbed is known as extinction length (Coluzzi, 2017).

With a short extinction length, the laser energy is absorbed maximally by the tissue, with no deep penetration and thus little risk of deep thermal damage. The laser energy penetrates deep into the tissue if the extinction length is long. Since CO_2 and erbium lasers are the two wavelengths that are better absorbed by tissue with a high water content, they have the shortest extinction period in mucosa and are the least likely to cause deep thermal harm if operating parameters are used correctly (Coluzzi, 2017).

CO_2 lasers, for example, have an extinction length of 0.03 mm in mucosa. If the proper operating settings are followed, Nd:YAG and diode lasers with extended extinction lengths in mucosa are likewise safe to use (Roenigk et al., 2016).

Operators who aren't properly trained to employ these wavelengths, on the other hand, run the risk of causing heat harm to the underlying tissue. Because carious lesions currently part more water than healthy tissue, and water is the primary absorber to laser energy, tissue laser systems can carefully extract damaged tooth structure (Manni, 2013, HOSSAIN et al., 1999). As they contact with the tooth surface, these devices show advantages over traditional high-speed hand pieces. Due to the bactericidal nature of laser radiation, there is no smear layer on laser dentin, and cavity preparation has been cleaned (Poli, 2017).

1.6.10 Laser applications in dentistry

1.6.10.1 Periodontal Laser Therapy

Lasers have largely been demonstrated to be bactericidal in previous research (Passanezi et al., 2015, Schwarz et al., 2008). Pathogens are destroyed with CO_2 and erbium laser by heating interior fluids and forcing the bacterium to collapse (Kreisler et al., 2002, AlMoharib et al., 2021). The photothermal effect is based on the absorption of laser power by tissues. With the correct settings, many nonsporulating bacteria, including anaerobes, can be killed at 50°C (Coluzzi and Convissar, 2007). Capillaries and lymphatics may be sealed with lasers, reducing edema and postoperative pain at the treated site (SAWISCH et al., 2015). Reduced edema, a higher infiltration of polymorphonuclear (leukocyte) neutrophils (PMNs), more fibroblasts, and better-organized collagen bundles all help wound healing (Reis et al., 2008).

1.6.10.2 Lasers in Implant Dentistry

Dental lasers' therapeutic function helped in enhancing implant dentistry's surgical, postsurgical, and prosthetic stages. In implant dentistry, both bony lasers erbium and soft tissue lasers (e.g., diode, CO_2) are used. In general, lasers help clinicians achieve better visibility of the surgical site by reducing bleeding (Coluzzi and Convissar, 2007, Dent, 2009, Swick, 2009), resulting in a shorter procedure time(Convissar, 2009). Complications and illnesses are greatly decreased by creating more hygienic environments both before and after surgery (Raffetto and Gutierrez, 2001). A CO_2 laser incision for a sinus raise, with an excellent postoperative result was anticipated. Reduced pain and swelling, as well as faster healing, are invaluable to the patient (Aoki et al., 2008, Convissar, 2009).

1.6.10.3 Lasers in Cosmetic Reconstruction and Fixed Prosthetics

It is possible to perform laser gingival troughing in conjunction with a laser gingivectomy simultaneously with impression taking, unlike blade or electrosurgical procedures. Crown-lengthening treatments have become increasingly influenced by esthetics as "smile enhancement" procedures (Alkhudhairy et al., 2020, Lee et al., 2007). An ideal emergence profile is required for treatments that fill gaps between teeth, including diastema closure, ovate pontic creation, and implant implantation (Terry, 2005). Laser treatment, according to the majority of dentists who perform gingival depigmentation, is the most effective and satisfactory procedure (Rosa et al., 2007).

1.6.10.4 Lasers in Endodontics

The advancement of modern laser delivery systems, thin metallic fibers, as well as novel endodontic tools, are among the new materials which improve endodontic treatment by laser. Method can now be used for the endodontic treatments are : Root canal cleansing and disinfection, root canal obturation, endodontic re-treatment, and apical surgery, and pulpotomy (Alamoudi, 2019, Goya et al., 2000).

1.6.10.5 Lasers in Restorative Dentistry

By using a proper laser wavelength, it became possible to the maintenance of healthy, mineralized dental tissue, with favored elimination of more cavities, more accuracy, and less germs in the beam cavity (Parker, 2015). Thermal conduction from rotary instruments can result in a conductive thermal elevation of more than 20°C over 37.4°C, which is a major cause of pulpal damage (Öztürk et al., 2004). When it comes to dental tissue laser irradiation, water-assisted midinfrared (IR) wavelengths cause explosive defragmentation, allowing the majority of the heat to escape and the only increase in pulp temperature less than 5°C (Karic et al., 2017, Oelgiesser et al., 2003).

1.6.10.6 Lasers in Pediatric Dentistry

Erbium lasers aided in the creation of a restorative procedure (drilling and filling), for the majority of pediatric patients inducated to receive dental caries treatment (Kumar, 2013). Erbium lasers are a non-invasive alternative to traditional methods that do not require local anesthesia. The most often utilized soft tissue lasers nowadays are carbon dioxide CO_2, neodymium-doped YAG, and diode lasers (Coluzzi, 2002).

1.6.10.7 Laser in preventive dentistry

Understanding how lasers interact with dental tissues is necessary for their use in preventative dentistry (Hibst et al., 2001). SEM and XRD examinations were used by Vahl to demonstrate the micro and crystallography alterations caused by laser irradiation. In vitro tests with a ruby laser (693.4 nm) found that the laser can warm the enamel surface, boosting demineralization resistance (Stern et al., 1969). Yamamoto fused enamel prisms at high energy levels (1 GW/cm^2) with a Nd:YAG laser , resulting in a significant increase in demineralization resistance (Yamamoto et al., 1974, Al-Maliky et al., 2020).

The melting of the enamel surface is controlled by a continuous CO_2 laser (250 – 1,000 J/cm^2) with a level of pulp heating. Lentz et al (1982) discovered ultra - structural and phases modifications on the tooth enamel treated with a constant CO_2 laser. Other researchers used low energetic levels and pulsed CO_2 laser irradiation (with a wavelength ranging from 9300 to 10600 nm) to test the effects on enamel and dentine created by CO2 laser irradiation (with a wavelength ranging from 9300 to 10600 nm)(Rechmann et al., 2020, Luk et al., 2019). The CO_2 laser, that wavelengths of 9300, 9600, or 10600 nm, was shown to be the primary device that could be used in prophylactic dentistry in all of these trials (Silva et al., 2020).

The selected CO_2 laser 10600 nm parameters used in the study (0.3 J/cm^2, 5 sec, 226 Hz, and 2,036 overlapped pulses) decreased enamel caries advancement by 81 % while causing subsurface heat damage visible by scanning electron microscopy (Esteves-Oliveira et al., 2009).

1.6.11 Enamel changes related to Laser:

Lasers are also used to avoid caries because of their strong interaction with dental hard tissues (Apel et al., 2005, Ana et al., 2006, Erkmen Almaz et al., 2021b) . Many studies was used of various lasers in the subject of decay prevention have been undertaken since then, including argon, CO_2, and Nd:YAG lasers (Moghadam et al., 2018).

Low-energy Nd:YAG laser dosages do not cause tissue melting and have been shown to improve permanent tooth enamel acid resistance (Afsheen et al., 2018). The application of sub-ablative energy from a Nd:YAG laser within pits and fissures without photo initiator substances resulted in a much more acid-resistant enamel without causing tooth tissue to melt (Pagano et al., 2020).

CO_2 and erbium lasers are commonly used in dental management and could be applied for caries prevention but with specific parameters to have best treatment results (Tagliaferro et al., 2007).

There has been a reduction in water content caused by Eribum, CO_2 and Nd:YAG lasers irradiation (Sasaki et al., 2002). In addition to their primary application of extract dental hard tissues, erbium lasers have been utilized to avoid demineralization. (Erkmen Almaz et al., 2021b, Afsheen et al., 2018). The decreased acid solubility of dental enamel after heating is explained by a number of theories. Dental enamel, for example, has been discovered to have a decreased water permeability following heating. There is more hydroxide and pyrophosphate in heated enamel than in unheated enamel, but less carbonate. (Erkmen Almaz et al., 2021b). The decrease of bonded carbonate because when enamel surface is warmed to 100–400°C is the most widely accepted idea for how laser treatment boosts enamel acid resistance (Moghadam et al., 2018).

1.6.12 Fluoride and laser effects on dental enamel:

Applying laser irradiation with topical fluoride treatment was suggested to increased caries resistance, and there are a number of mechanisms at work to explain why caries resistance improves following laser and fluoride therapy on tooth. Laser irradiation coupled with fluoride treatment thought to improves creative fluoride absorption (Ana et al., 2006).

The association of laser irradiation with fluoride has demonstrated the best results in the inhibition of caries development. The combined treatment of laser irradiation with fluoride propitiates an expressive fluoride uptake, reducing the progression of caries-like lesions, and this treatment is more effective than laser or fluoride alone (Ana et al., 2006).

Laser-irradiated enamel was proposed to maintains fluoride for longer than unlased enamel (Nammour et al., 2005, Ana et al., 2006). The target was to initiate the conversion of hydroxyapatite to fluorapatite through laser irradiation and fluoride treatment. Due to the heating generated by CO_2 9600 nm wavelength light irradiation, fluoride can be integrated into the melting layers of tooth enamel (Silva et al., 2020). Although it's been proposed that utilizing the Er:YAG laser in combination with topical fluoride can help prevent acid demineralization of permanent tooth enamel, more research is indicated considering laser parameters and type (Chen et al., 2009).

Controversial studies demonstrated that fluoride administered to the enamel following or before laser irradiation to reduce caries susptibility (Apel et al., 2004, Apel et al., 2002). Furthermore, depending on whether the laser treatment occurs before or after the fluoride administration, results explained every technique but agree on a combined of laser irradiation and topical fluoride treatment reduces caries lesion progression (dos Santos et al., 2002, Hicks et al., 2004). Formation of protective barrier against cariogenic attack was explained by some modification or changes in the structure or surface of tooth enamel interaction with eribium laser (Ana et al., 2012b).

1.7 Literature review

The improvement of enamel microhardness and acid resistance using different types of lasers was evaluated by varies studies as appear in (Table 1.3)

where:

- The first column shows the author's name and the year of publishing.
- The second column displays the type of laser used in the study.
- The third column represent the laser parameters
- The fourth column represents the test types
- The last column displays the conclusion of this study that related to the present study.

Table (1.3): Literature review

Author/ Year	**Type of laser Laser**	**parameters**	**tests**	**Conclusion**
Kenneth Luk et al 2021)Luk et al., 2021)	10,600 nm carbon dioxide laser	P = 0.1-2800 w Pulse energy=0.00025-2000 mj Spot size=$8.7*10^{-8}$ – 0.05 cm^2 Frequency = 1-226 Hz Fluence=0.3-28.6	SEM EDS	The use of power or energy meter to validate the pre-set machine setting is important. although fluence is the most referenced parameter in research conclusions, irradiation techniques are important for producing the outcome effect.
Erkmen et al 2021 (Erkmen Almaz et al., 2021b)	Er. Cr: YSGG laser	Power= 0.25-0.75 w PPR =20 Hz	FTIR analyzes RAMAN analyzes SEM Thermocouple device	the temperature rise during Er. Cr:YSGG laser irradiation for the prevention of primary enamel demineralization exhibited a positive correlation with the laser output power level (0.50 W ≤ power ≤ 0.75 W at the frequencies of 20 Hz).
AlShamrani et al 2021 (AlShamrani et al., 2021)	Er.Cr:YSGG laser	Power=0.5 W repetition frequency= 20 Hz, pulse duration=60 µs, exposure time= 10 s air=11% water= 0%	Microhardness	The results showed that application of fluoride alone as well as the combination of laser irradiation followed by fluoride application were the only surface treatments that increased the resistance of the tooth enamel to the progression of erosion and improved the microhardness of the enamel surface.

Nagehan Yilmaz et al 2020 (Yilmaz et al., 2020)	Er.Cr:YSGG laser	power =0.5 W PRR= 20 Hz water =60%, air=40% pulse energy =25 mJ fluence =8.84 J/cm^2 pulse duration= 60 µs	Microhardness SEM XRD	the remineralization agents produced increased surface microhardness values from DM to RM but it was not statistically significant in ROCS® medical mineralgel. The combined use of remineralization agents with a laser did not cause significant differences in remineralization.
da Silva et al 2019 (da Silva et al., 2019)	Er,Cr:YSGG laser	Power= 0.25 - 0.75W, PPR=20 Hz Energy density= 2.8, 5.7, 8.5 J/cm^2 power density=56, 1136, 1704 W/cm^2	optical profilometer SEM	Association of the Er,Cr:YSGG laser in parameter 2 with fluoride was the only treatment capable of controlling the progression of enamel erosion.
A. Belcheva et al 2018 (Belcheva et al., 2018)	carbon dioxide 10,600-nm laser	average power= 0.73 W time on= 100 µs time off= 40 ms tip-to-tissue distance=20 mm tip diameter= 700 µm energy density = 5 J/cm^2	Vickers microhardness SEM	Microhardness values found in the current study showed a significant difference between lased and unlased groups as well as between sample groups with and without fluoride application. The laser-irradiated and fluoride-treated samples (FL group) showed the least diminution in enamel surface microhardness.
de Oliveira et al 2017 (de Oliveira et al., 2017)	Er.Cr:YSGG Laser	power = 0.10–1.00 W frequency=5–75 Hz	Profilometer roughness Microhardness	the irradiation of Er,Cr:YSGG laser with different parameters of power and pulse frequency settings may alter enamel surface and erosive resistance differently. Pulse frequency of 30 Hz and power of 0.50 W was considered the best parameter to prevent enamel acid erosion.
Santos Jr et al 2014 (Santos Jr et al., 2014)	Er, Cr:YSGG and Nd:YAG Lasers	Er, Cr:YSGG laser (8.92 J/cm2, 0.5 W, 20 Hz, 30 seconds) Nd:YAG laser (84.9 J/cm2, 0.5 W, 10 Hz, 30 seconds)	Microhardness SEM polarized light microscopy	Laser irradiation, with or without applying five percent sodium fluoride, was not capable of increasing the enamel white spot lesions' acid resistance.
P.A. Ana et al 2012 (Ana et al., 2012b)	Er.Cr:YSGG Laser	power= 0 to 6 W energy density=2.8, 5.6 and 8.5 J/cm^2 Pulse width =140 µ s	Microhardness SEM	The findings suggest that laser treatment at 8.5 J/cm 2 was able to decrease hardness loss, even though no additive effect with APF was observed. In

		repetition rate =20 Hz spot size =430 µm		addition, laser treatment increased the formation and retention of CaF 2 on dental enamel

1.8 Aims of the study

1- Evaluation of Enamel Roughness and SEM after different laser parameters (CO_2 and Er.Cr:YSGG lasers).
2- Evaluation of laser treatment effect on enamel microhardness using Vickers test.
3- Comparison of laser effect with or without fluoride application on enamel microhardness after pH cycling

Chapter Two
Material and Method

CHAPTER TWO

MATERIAL AND METHOD

2.1 Introduction

This chapter includes a detailed description of all the materials and equipment used in the present study, with the methods used to perform this study.

2.2 Materials:

- Acetic acid CH_3COOH 99.7% (GILLMAN, Australia).

- Acetone 9% (Nada, Iraq).

- Acidulated phosphate fluoride (ALPH-PRO APF, DENTAL TECHNOLOGIES, USA)

- Acrylic powder and monomer (DMP Ltd, Made in E.U.).

- Adhesive tape (Eurotape).

-Calcium chloride $CaCl_2$ (Riedel-dehaenag Seelez, Germany).

-Calcium nitrate $Ca\ (NO_3)_2$ (BDH Chemicals Ltd. Poole, England).

-Cotton and gauze (China).

- Cutting disk (China).

-Disinfecting agents (Dettol and Spirit) (Al-Mansour)، Iraq.

-Disposable gloves (Master guard, Malaysia).

-Nail varnish (Super-Fast, Germany).

-Potassium chloride (KCl) (BDH Lab. Reagents, England).

-Potassium phosphate (KH_2PO_4) (BDH Lab. Reagents, England).

-Prophylactic rubber cups (Try Care, UK).

-Ruler (China).

- Sound, non caries extracted human teeth.

- Thymol crystals (BDH, England).

- Tweezers (Medident, Germany).

- De-ionized water (Iraq).

-Disposable Jar (China).

-Non fluoridated pumice (Quayle dental, England).

2.3 Equipment's and instruments:

- Anterior forceps (Germany)

- Optical microscope with Digital camera (OLYMPUS/BX51, Korea)

- CNC auto machine (custom made Iraq)

- Vickers hardness tester (Model: TH715, SN: 0003, Beijing Time High Technology Ltd, China).

- Energy Dispersive Spectroscopy (PentaFET Precision, Oxford Instruments, X-act, Model: AIS2300 SN:112544, USA).

- Er.Cr:YSGG laser 2780nm (Waterlase iPlus; Biolase, Irvine, CA, USA)

- Low speed hand-piece engine (W&H, Austria).

- pH meter (Consort, model, 832, Belgium).

- Stereo microscope (photographic with unit control, Olympus HBH, Japan)

- Thermocouple device (ASWAR, China)

- Triple air syringe of a dental chair unit (China).

- Water bath (HH-2 Numerical Show Constant Temperature Water-Bathing Boiler, China).

-Fractional CO_2 laser system (CO_2 Fractional Laser Brochure, JHC1180, China)

- Dental survyor (DENTAURUM paratherm)

-Scanning electron microscope (Model: AIS2300 Multi-function SEM system, SN: SEM:112544, USA).

2.4 Sample

In this investigation, dental samples were collected 40 maxillary first premolars and mandibular third molar extracted from Iraqi patients 14-28 years old due to orthodontic treatment and teeth impaction. These teeth were collected from the followings: Ministry of health specialist dental health centers and private clinics in Baghdad city.

Figure (2.1): Teeth samples and sectioning

All soft tissues were removed, and the teeth were polished with a fluoride-free, ultra-fine pumice and put the sample in ultrasonic cleaner. Each tooth was sectioned mesiodistally in two parts. To avoid tooth drying, the teeth were dipped in 0.1 % thymol as an antibiotic and antiviral disinfectant, then immersed in distilled water.

2.5 Pilot study

To estimate the most suitable laser parameters that induce surface modification without adverse effect, a pilot study was conducted. Although Fractional CO_2 and Er.Cr:YSGG laser irradiation can be use in dentistry safely.

Different laser parameters were tested considering laser power (0.25 w,0.5 w, 0.75 w and 1.0 w), PPS 20 Hz, 10 % air and (1%, 10%) water for Er.Cr:YSGG laser and different power (1 w , 2 w , 3w and 4w) , plus duration 10.0ms , interval 1.0 ms, distance 0.2 mm and scan modes was normal for Fractional CO_2 laser.

After irradiation of each sample, it was examined under light microscope to see the morphologic changes and surface texture, as show in figure (2.1) and a profilometer roughness measurement. According to the pilot study test, final laser parameters were (0.25 w, 0.50 w), PPS 20 Hz, Air: 10 % and Water: 1 % for Er.Cr:YSGG laser and power 1W , plus duration 10.0ms , interval 1.0 ms, distance 0.2 mm and normal scan modes for Fractional CO_2 laser.

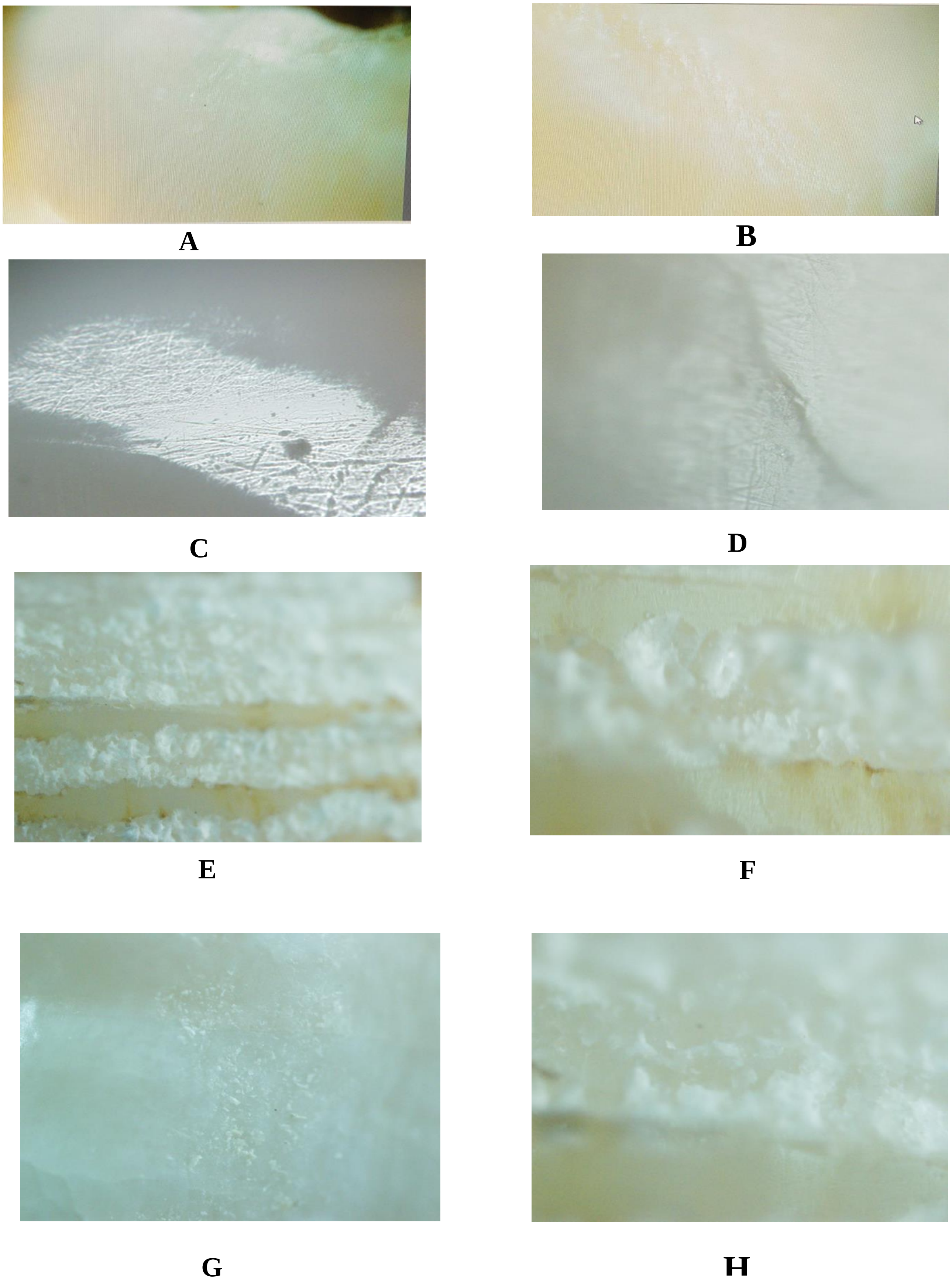
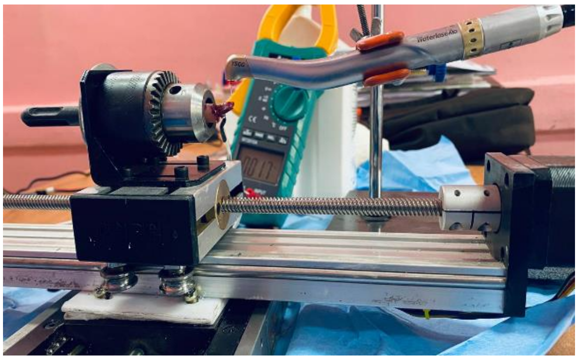


Figure (2.2): Light microscope image to see the morphologic changes and surface texture after lasing Aand B before, for Er.Cr:YSGG(0.25W(C), 0.5W(D), 0.75W (E),1.0 W (F), for CO_2 1W (G) and 2 W (H).

Table (2.1): pilot study for Er.Cr:YSGG and CO_2 laser for different parameters

Laser type and power	Microhardness		roughness	
	Before laser treatment	After laser treatment	Before laser treatment	After laser treatment
0.25 W for Er.Cr:YSGG	259	297	2.532	2.563
0.5Wfor Er.Cr:YSGG	243	324	2.319	2.335
0.75Wfor Er.Cr:YSGG	256	349	2.429	2.962
1.0w for Er.Cr:YSGG	249	382	2.469	3.326
1 W for CO_2	244	299	2.637	2.676
2 Wfor CO2	241	309	2.413	2.499
3 Wfor CO2	253	371	2.762	3.563
4 W for CO2	257	391	2.368	3.695

In order to achieve morphological or chemical changes in enamel surface that enhance caries resistance of the tooth without adverse effect, measuring the changes in the temperature in the pulp due to laser application, thermocouple device was set to measure temperature in the pulp cavity during lasing, as show in figure (2.3). Pulp temperature was not increased over 1C^{o} therefore there no hazardous effect due to laser use.



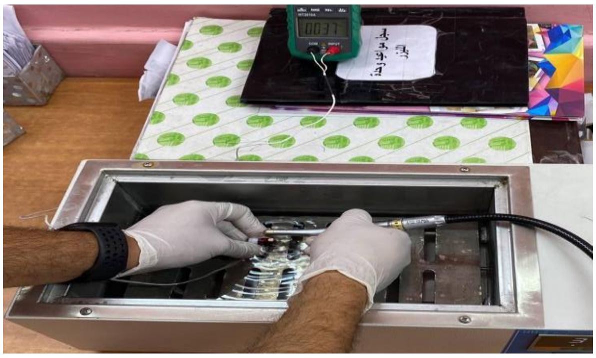


Figure (2.3): Thermocouples device to measurement heat temperature in the tooth during laser irradiation for Er.Cr:YSGG laser.

2.6 Study groups

80 samples obtained were randomly assigned to one of eight groups (N = 10), each with a different surface treatment as follow:

Group (G1): Control teeth, no there any treatment.

Group (G2): Teeth treated with acidulated phosphate fluoride 1.23% only.

Group (G3): Teeth treated with 0.25W, 20Hz, 1% water, 10% air Er.Cr:YSGG laser only.

Group (G4): Teeth treated with 0.5W, 20Hz, 1% water, 10% air Er.Cr:YSGG laser only.

Group (5): teeth treated with 1 W, duration 10.0 ms , interval 1.0 ms , distance 0.2 mm and scan modes is normal Fractional CO_2 laser only.

Group (G6): Teeth treated with 0.25W 20Hz, 1% water, 10% air Er.Cr:YSGG laser and Acidulated Phosphate fluoride 1.23%.

Group (G7): Teeth treated with 0.5W 20Hz, 1% water, 10% air Er.Cr:YSGG laser and Acidulated Phosphate fluoride 1.23%.

Group (8): teeth treated with 1 W, duration 10.0 ms, interval 1.0 ms , distance 0.2 mm and scan modes is normaol Fractional CO_2 laser and Acidulated Phosphate fluoride 1.23%.

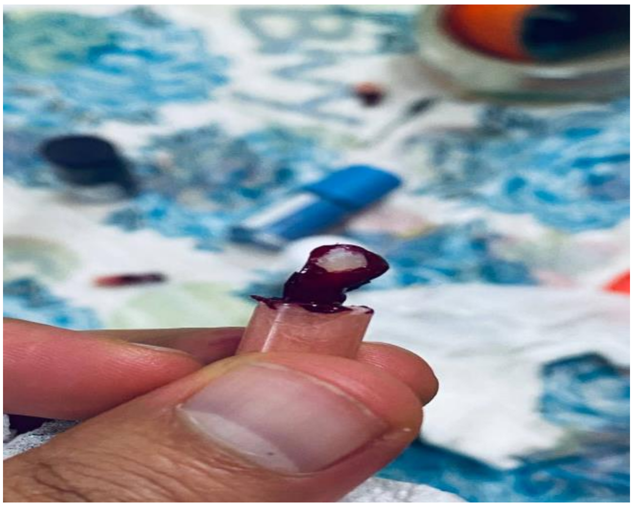
2.7 Enamel surface preparation:

The teeth sample were adjusted in the acrylic mold (size of the mold used was 14 * 10 mm) by using a cap for standardization. Then the teeth sample was fixed in the dental surveyor with cutting disk hold in low-speed handpiece (60-70 rpm) for minimal surface modification to get a flat enamel surface. A reflected light microscope was used to examine the teeth and look for any abnormalities on the surface (to be discarded). A position of circular window of 4 mm in diameter was standardized in the middle and occlusal third of the buccal and lingual surface of the samples. By using lethal hold punch on the adhesive tape a circle shape with 4*4 mm diameter was made, to standardize the area included in the test for all the samples. Later a nail varnish with acid resistance was used to paint all surface of the tooth. Then the circular tape was removed leaving a window of enamel on the sample surface. The teeth were coated with acid resistant varnish before laser irradiation, leaving two 4*4 mm wide windows buccally and lingually, as show in figure (2.4).

A

B

C



D

Figure (2.4): A: Dental survyor, B: Acrylic material using for mold preparation, C: sample molding, D: final sample.

2.8 Lasing conditions

A Lasing was done at University of Baghdad, institute of Laser for post-graduat studies

- Er,Cr:YSGG dental laser device (figure 2.5, A) was used to perform the laser irradiation. It is of 2780 nm, a pulse width of 60 µs, a repetition rate of 20 Hz and 0.25 or 0.5 W of power. The energy is delivered via a fiberoptic system with a sapphire terminal a width 600 µm and a length of 6 mm (MZ6 tip), used with 10% air and 1% water spray. The laser handpiece was stabilized with the clamp during lasing while the tooth was attached to a CNC machine during laser irradiation. The tip of the fiber was positioned at a standard distance of 2 mm from the enamel surface, and laser irradiation was performed in all areas of unpainted enamel surface.

- Fractional CO2 laser: This laser emits invisible infra-red (IR) laser at 10600 nm wavelength, figure (2.5, B).

Laser power was estimated to be 1.0 W, energy 10 mj, duration time 10.0 ms, interval 1.0 ms, distance 0.2 mm, scan mode was single scan and Normal mode.

A **B**

Figure (2.5): A: Er.Cr:YSGG Laser , B: Fractional CO_2 Laser.

2.9 Surface treatment

2.9.1 Fluoride application

No surface treatment in Group 1 (control group) was done. Acidulated phosphate fluoride (group 2) (1.23%, (0.79% from sodium fluoride and 0.44% from hydrogen fluoride) in 0.1 molar phosphoric acid) figure (2.6) for 4 minutes with a cotton bud, then the gel was removed using cotton rolls according to the manufacturer's recommendations.

Figure (2.6): Acidulated Phosphate Fluoride 1.23%.

2.9.2 Laser irradiation

Group 3 samples were exposed to a pulsed Er, Cr:YSGG laser with the following parameters: 0.25 W power, 20 Hz repetition frequency, 60 μs pulse duration, and 10 s exposure period (10 % and 1 % of air pressure and water level, respectively). Group 4 is similar to Group 3 but with a different power level (0.5 W, 20Hz, 10 % air, 1 % water). For group (5) were irradiated with Fractional CO_2 laser 1 W, duration 10.0 ms, interval 1.0 ms, distance 0.2 mm and scan modes were normal.

2.9.3 Laser treatment and fluoride

For group 6, 10 seconds of laser irradiation was performed at 0.25 W, 20 Hz, 10% air, 1% water followed by 4-minute treatment with fluoride gel. For group 7, laser irradiation was performed at 0.5 W, 20 Hz, 10% air, 1% water followed by 4-minute treatment with fluoride gel. For group 8 ,1 W, duration 10.0 ms, interval 1.0 ms, distance 0.2 mm and scan modes is normal fractional CO_2 laser followed by 4 -minute treatment with fluoride gel.

2.10 PH Cycling

The carise like lesion was induced on enamel surface according to Albazzaz (Al-Bazzaz, 2017) therefore the pH cycling was caried out. This procedure was done by the preparation of demineralizing and remineralizing solutions and adjustment of Ph.

A- Demineralizing solution: This solution comprised of:

1) 2.0 mMol/L potassium phosphate

2) 1.0 mMol/L calcium chloride

3) 0.075 Mol/L acetic acid

The pH was adjusted to 4.5 by pH meter at 37˚C.

B-Remineralizing solution: This solution comprised:

1) 0.9 mMol/L potassium phosphate

2) 150 mMol/L potassium chloride

3) 1.5 mMol/L calcium nitrate

The pH was adjusted to 7, at 37˚C.

The procedures of Remineralization and Demineralization were done using the following steps:

1- Micro hardness of enamel was measured for normal (sound) enamel.
2- Teeth were treated according to the groups design; irradiated with Er.Cr:YSGG or Fractional CO_2 laser in a specific lasing parameters and/ or treated with Acidulated phosphate fluoride and/or combination between laser and fluoride.
3- Micro hardness of enamel was measured after each samples treated.
4- Each tooth was dipped in demineralizing solution for 6 hours at 37˚C in the water bath.
5- Then each tooth was withdrawn and washed for one minute with running de-ionized water.
6- After that, teeth samples were dipped on remineralizing solution for 18 hours at 37˚C in the water bath.
7- Each tooth sample then was withdrawn and washed for one minute with running de-ionized water before repeating the cycle another time.
8- The cycling of Demineralization- Remineralization finish with 24 hours, repeated for 8 days.
9- Then the teeth sample were dipped in remineralization solution for 2 days.
10- Sample then were washed with de-ionized water.

11- Enamel microhardness was measured after the pH cycling procedure.

2.11 Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS):

Scanning electron microscope and energy dispersive spectroscopy were carried out in Al-Nahrian University, College of Science, department of Physics.

2.11.1 Scanning Electron Microscope (SEM):

The morphological changes in enamel surface were studied using a scanning electron microscope (SEM) with a focused beam of electrons. the electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the detected signal to form a picture(Laws et al., 2022).

Representative for SEM sample preparation, one specimen from each group was chosen. Using a vacuum system coating machine, the selected teeth were gold coated.

2.11.2 Energy Dispersive Spectroscopy (EDS):

To measure the changes in the elements' weight % of the enamel after being irradiated with different laser parameters and fluoride The researcher employed an EDS (energy dispersive X-ray spectrometer). Furthermore, each sample's Ca/P ratio was calculated as a percentage of total weight.

Figure (2.7): Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS)

2.12 Micro hardness test

A micro hardness tester was used to determine Vickers micro hardness, which includes a diamond indenter with a square base and a 400x magnification high-resolution optical microscope, as show in the figure (2.8).

A load of 1000 g (9.8 N) was applied for 15 seconds at three distinct locations on each sample during the measurement objective as a baseline before laser irradiation after laser irradiation and after demineralization – remineralization cycling. The average of three measurements was used to establish the micro hardness value of each sample (Karlinsey et al., 2011).

Figure (2.8): Vickers micro hardness device

2.13 Roughness

A profilometer was used to assess surface roughness by a single blinded examiner, as show in the figure (2.9). To ensure reliable profile measurements, three spots were first designated. With a cutoff of 0.25 mm (kc) and a speed of 0.1 mm/sec. The surface roughness of each unit was measured, and the mean roughness value of the three profiles was calculated in two phases: baseline, after irradiation (Barac et al., 2015).

Figure (2.9): Profilometer roughness device

2.14 Statistical Analysis:

The results obtained in this research were analyzed statistically with SPSS program version 20. The statistics include two types:

1- Descriptive Statistics: that include

-mean

- standard error (SE),

-standard deviation (SD)

- maximum and minimum values.

-Graphical presentation by diagram.

2- Inferential Statistics:

• Shapiro-Wilks's test to determine if the data are normally distributed or not. The data are normally distributed when $p>0.05$.

• One Way Analysis of Variance (One-Way ANOVA) and TUCKY HSD test were a used to make analysis or to study if there are statistically significant differences between means of two or more unrelated groups.

Level of significance was determined as follow:

$P > 0.05$ not significant (NS)

$P \leq 0.05$ significant (S)

$P < 0.01$ highly significant (HS)

Chapter Three

Result , Discussion , Conclusion and Suggestion

CHAPTER THREE

RESULTS, DISCUSSION, CONCLUSION AND SUGGESTION

3.1 Introduction

Teeth samples in this study consisted of 80 samples from maxillary first premolars and mandibular molar. They were divided in control and study groups for Microhardness, Roughness, Scanning Electron Microscope examination (SEM) and Energy Despersive Spectroscopy (EDS) analysis.

3.2 Microhardness Test:

3.2.1 Data distribution

Means of microhardness values for all groups and treatment types are shown in figure (3.1)

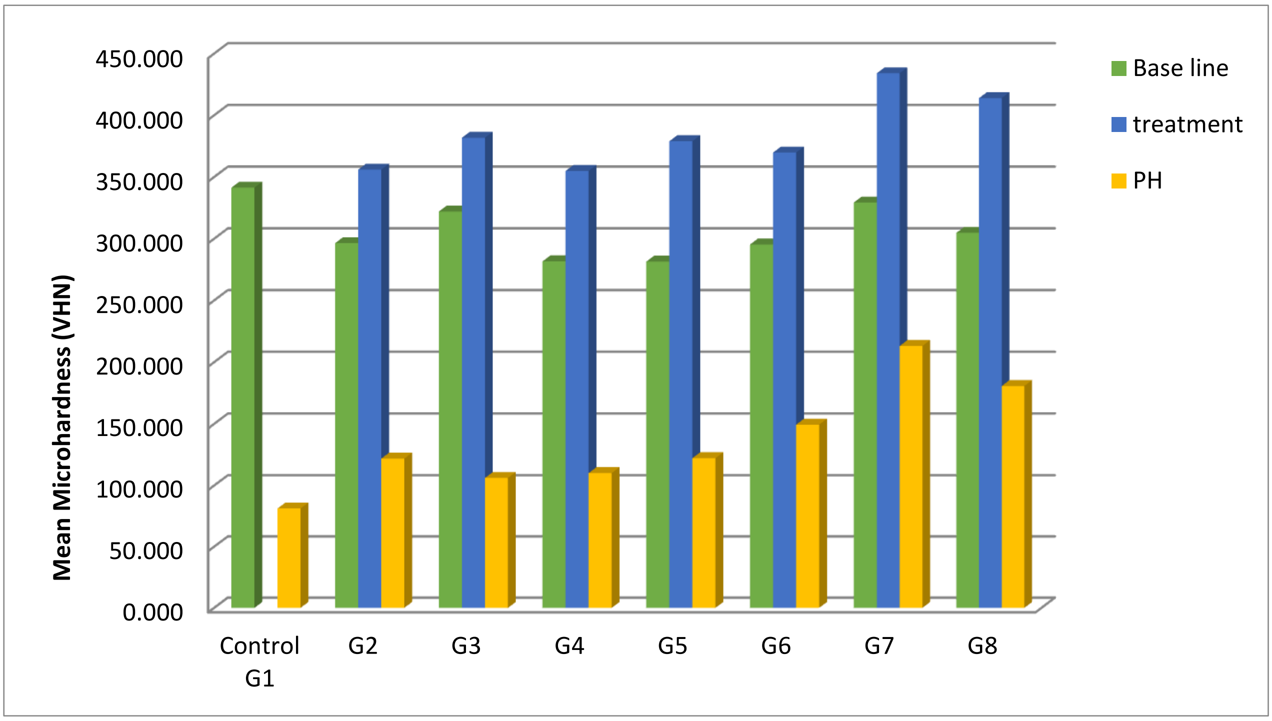


Figure (3.1): Bar-chart shows means of microhardness for all groups.

It seems that all the treatment increased the mean values of the enamel microhardnes, also all the groups showed reduction of the microhardness following Ph cycling.

First, Shapiro wilk test was made to evaluate data distribution normality, for all group (experimental) in base line, after treatment and after Ph cycling table (3.1). It shows a non-significant differences and normal data distribution.

Table (3.1): Shapiro-Wilk test for normality distribution of data in all groups.

Tests of Normality					
Treatment	Groups	Shapiro-Wilk			
		Statistic	df	P value	
Base Line	G1	0.957	10	0.751	NS
	G2	0.968	10	0.876	
	G3	0.955	10	0.726	
	G4	0.904	10	0.245	
	G5	0.923	10	0.385	
	G6	0.928	10	0.429	
	G7	0.960	10	0.784	
	G8	0.945	10	0.609	
After treatment	G2	0.920	10	0.356	
	G3	0.936	10	0.507	
	G4	0.852	10	0.061	
	G5	0.969	10	0.879	
	G6	0.953	10	0.702	
	G7	0.947	10	0.636	
	G8	0.949	10	0.661	
After PH	G1	0.975	10	0.931	
	G2	0.852	10	0.061	
	G3	0.877	10	0.121	
	G4	0.958	10	0.759	
	G5	0.945	10	0.605	
	G6	0.953	10	0.701	
	G7	0.968	10	0.875	
	G8	0.942	10	0.574	

A table (3.2) for descriptive statistics is including: -mean, standard deviation, standard error, minimum and maximum values for microhardness in all groups as well as ANOVA test.

Table (3.2) Descriptive and inferential statistical test of Microhardness among groups and treatment.

Groups		Base line	Laser	After PH	Statistics	P value *
Control G1 #	Min.	303.400		58.530	54.969	0.000
	Max.	376.730		109.800		
	Mean	301.367		81.209		
	±SD	21.920		14.285		
G2^	Min.	203.900	303.700	107.100	138.234	0.000
	Max.	383.800	411.100	150.700		
	Mean	296.380	355.960	121.847		
	±SD	53.907	39.274	15.845		
G3 ^	Min.	224.900	308.100	88.850	190.285	0.000
	Max.	387.600	456.900	138.000		
	Mean	322.040	381.790	106.184		
	±SD	47.456	48.296	17.031		
G4 ^	Min.	234.200	291.900	91.630	153.341	0.000
	Max.	356.400	398.300	135.600		
	Mean	281.677	354.940	110.156		
	±SD	38.860	40.019	13.242		
G5 ^	Min.	200.300	300.900	98.640	176.119	0.000
	Max.	340.700	447.500	147.100		
	Mean	281.440	378.960	122.200		
	±SD	43.524	44.475	17.121		
G6 ^	Min.	205.900	303.000	119.400	126.608	0.000
	Max.	348.200	456.100	186.000		
	Mean	295.336	369.790	149.350		
	±SD	42.129	49.002	17.795		
G7 ^	Min.	271.500	357.100	173.200	140.417	0.000
	Max.	390.900	496.700	241.100		
	Mean	329.290	434.020	213.139		
	±SD	39.650	43.046	20.877		
G8 ^	Min.	238.000	345.500	129.000	155.676	0.000
	Max.	397.500	501.700	227.800		
	Mean	304.780	413.890	180.650		
	±SD	46.249	48.925	33.797		
ANOVA (F)		2.124	4.350	48.057		
P value		0.052 NS	**0.001 Sig.**	**0.000 Sig.**		

#=Paired T test, ^=Repeated Measure ANOVA, NS=not significant Sig.=Significant at $p<0.05$.

It is shown that in all groups and treatments there was significant differences according to the ANOVA test except comparison between groups at the baseline. Further analysis was done using Tukey HSD to estimate differences in microhardness means between different groups considering treatments, table (3.3).

Table (3.3) Pairwise Comparisons of Microhardness between groups using Tukey HSD after treatment.

Multiple Comparisons/ Dependent Variable: MH Laser			
Tukey HSD			
(I) Groups	(J) Groups	Mean Difference (I-J)	P value
G2	G3	-25.830	0.202870
	G4	1.020	0.959634
	G5	-23.000	0.256203
	G6	-13.830	0.493361
	G7	-78.060	0.000245*
	G8	-57.930	0.005338*
G3	G4	26.850	0.185829
	G5	2.830	0.888331
	G6	12.000	0.552103
	G7	-52.230	0.011539*
	G8	-32.100	0.114787
G4	G5	-24.020	0.235932
	G6	-14.850	0.462168
	G7	-79.080	0.000207*
	G8	-58.950	0.004626*
G5	G6	9.170	0.649362
	G7	-55.060	0.007918*
	G8	-34.930	0.086715
G6	G7	-64.230	0.002154*
	G8	-44.100	0.031707*
G7	G8	20.130	0.319773

*,Sig.=Significant at p<0.05.

Results showed that Microhardness values was not significantly different between lasers and fluoride groups while G6, G7, G8 (combination treatments) generally showed significant increase when compared with other treatments. (G7 with G2, G3, G4, G5 and G6; G8 with G2, G4 and G6).

Regarding the change in microhardness values between baseline and treatments table (3.4).

Table (3.4): Pairwise Comparisons of Microhardness between treatment by groups Using paired t-test.

Groups	Treatment		Mean Difference	P value*
G2	Base line	After laser	-59.580	**.000**
G3	Base line	After laser	-59.750	**.000**
G4	Base line	After laser	-73.263	**.000**
G5	Base line	After laser	-97.520	**.000**
G6	Base line	After laser	-74.454	**.000**
G7	Base line	After laser	-104.730	**.000**
G8	Base line	After laser	-109.11	**.000**

*=significant at p <0.05.

To test the effect of Ph cycling on the microhardness values between groups, multiple comparison was made. Results in table (3-5) show that Microhardness values recorded significant difference when compare each group with other except when compare G2 with G5, G4 and G3 was not significant. Comparison between laser groups showed non-significant differences between G3 with G4 and G5 as well as G4 with G5. Anon-significant difference was also recorded between G7 and G8.

Table (3.5) Multiple Comparisons of Microhardness after PH between groups using Tukey HSD.

(I) Groups	(J) Groups	Mean Difference (I-J)	p value
G1	G2	-40.638	0.000301*
	G3	-24.975	0.054826^
	G4	-28.947	0.004687*
	G5	-40.991	0.000500*
	G6	-68.141	0.000001*
	G7	-131.930	0.000000*
	G8	-99.441	0.000044*
G2	G3	15.663	0.622149^
	G4	11.691	0.839677^
	G5	-0.353	1.000000^
	G6	-27.503	0.044119*
	G7	-91.292	0.000000*
	G8	-58.803	0.006309*
G3	G4	-3.972	1.000000^
	G5	-16.016	0.644443^
	G6	-43.166	0.000782*
	G7	-106.955	0.000000*
	G8	-74.466	0.000710*
G4	G5	-12.044	0.854971^
	G6	-39.194	0.000931*
	G7	-102.983	0.000000*
	G8	-70.494	0.001349*
G5	G6	-27.150	0.062372^
	G7	-90.939	0.000000*
	G8	-58.450	0.006752*
G6	G7	-63.789	0.000025*
	G8	-31.300	0.347968^
G7	G8	32.489	0.343460^

^=not significant, *=significant at $p<0.05$.

Table (3.6) shows comparison between baseline and after Ph cycling for each group indicated significant reduction of microhardnes reduction using paired t-test.

Table (3.6): Pairwise Comparisons of Microhardness between baseline and after Ph cycling using paired T-test.

Pairwise Comparisons of Microhardness between treatment by groups Using paired T-test *				
Groups	Treatment		Mean Difference	P value*
G2	Base line	After PH	174.533	**.000**
G3	Base line	After PH	215.856	**.000**
G4	Base line	After PH	171.521	**.000**
G5	Base line	After PH	159.240	**.000**
G6	Base line	After PH	145.986	**.000**
G7	Base line	After PH	116.151	**.000**
G8	Base line	After PH	-109.110	**.000**

3.3: Roughness Test

3.3.1 Data distribution

First, Shapiro Wilk test was applied to test normality of the data in all groups, in base line and after treatments table (3.7)**.**

Table (3.7) Shapiro Wilk test of roughness among groups and treatments.

Tests of Normality				
Periods	Groups	Shapiro-Wilk		
		Statistic	df	P value ^
Base line	G2	0.883	10	0.140
	G3	0.893	10	0.185
	G4	0.859	10	0.075
	G5	0.947	10	0.628
	G6	0.914	10	0.312
	G7	0.935	10	0.496
	G8	0.909	10	0.272
After	G2	0.900	10	0.217
	G3	0.944	10	0.604
	G4	0.884	10	0.145
	G5	0.927	10	0.421
	G6	0.957	10	0.755
	G7	0.924	10	0.394
	G8	0.884	10	0.145

^=not significant at p>0.05.

Results indicated normal distribution of the data therefore; further analysis was done using descriptive and inferential statistics.

3.3.2 Descriptive statistics

Below is a table (3.8) for descriptive statistics including mean, standard deviation, standard error, minimum and maximum values of the roughness in all groups.

Table (3.8): Descriptive and ANOVA test of roughness among groups.

Groups		Mean	±SD	±SE	Minimum	Maximum	F-test	P value
Base line	G2	2.525	0.263	0.083	2.103	2.818	1.083	0.383 (NS)
	G3	2.354	0.269	0.085	2.104	2.765		
	G4	2.512	0.205	0.065	2.116	2.759		
	G5	2.433	0.221	0.070	2.035	2.725		
	G6	2.627	0.276	0.087	2.313	2.986		
	G7	2.541	0.403	0.127	2.019	3.314		
	G8	2.400	0.310	0.098	1.793	2.873		
After treatment	G2	2.590	0.237	0.075	2.168	2.868	1.705	0.135 (NS)
	G3	2.363	0.271	0.086	2.104	2.780		
	G4	2.520	0.209	0.066	2.130	2.759		
	G5	2.461	0.228	0.072	2.048	2.804		
	G6	2.643	0.271	0.086	2.313	3.011		
	G7	2.699	0.392	0.124	2.168	3.445		
	G8	2.473	0.318	0.101	1.886	2.890		

In the Baseline all groups are not significant difference in roughness value while after treatment, although findings above show that higher value of roughness find in G7 and G2 respectively followed by G6 and G4 respectively while the least value finds in G8, G5 and G3 respectively, but with no significant difference between groups. Figure showed (3.2) changing in Roughness value at base line and after treatment

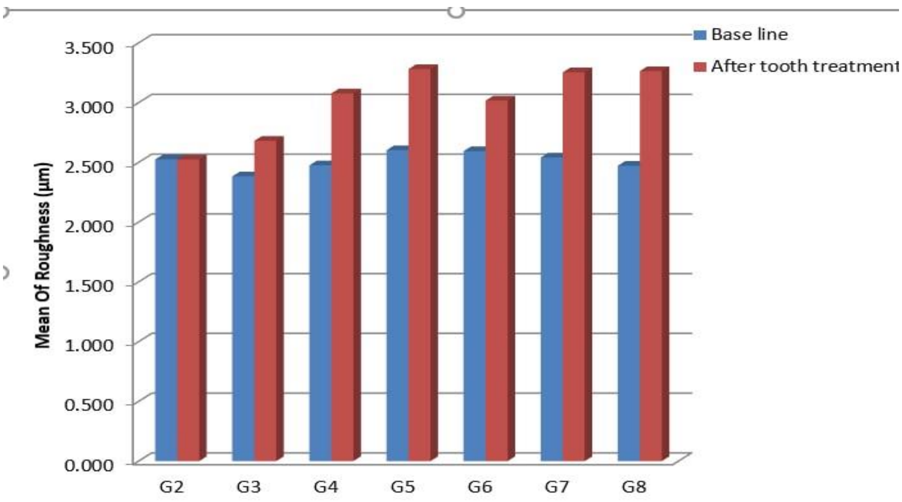


Figure (3.2): Diagram changing in Roughness value at base line and after treatment

Table (3.9) Descriptive and statistical test of roughness change from baseline to treatment by groups.

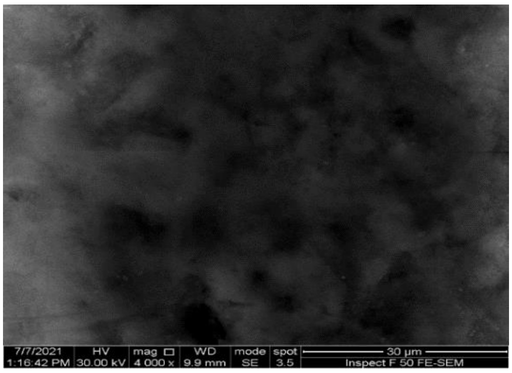
Groups	Baseline		Treatment		ΔMean	Paired T test	P value
	Mean	±SD	Mean	±SD			
G2	2.525	0.263	2.590	0.237	-0.06510	3.607	0.052
G3	2.354	0.269	2.363	0.271	-0.00820	2.623	0.140
G4	2.512	0.205	2.520	0.209	-0.00820	1.857	0.192
G5	2.433	0.221	2.461	0.228	-0.02770	2.288	0.144
G6	2.627	0.276	2.643	0.271	-0.01570	2.589	0.140
G7	2.541	0.403	2.699	0.392	-0.15840	1.279	0.233
G8	2.400	0.310	2.473	0.318	-0.07330	2.723	0.138

^=not significant at $p>0.05$, *=significant at $p<0.05$.

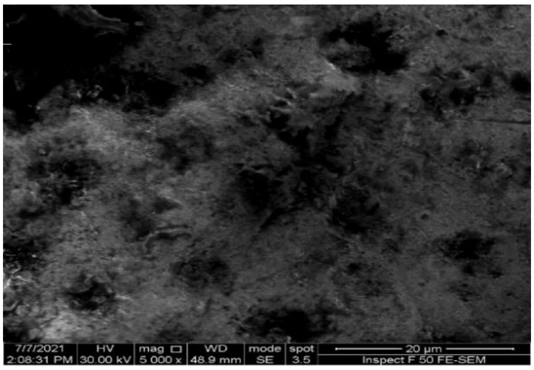
Findings in table (3.9) show that roughness in all groups finds to be increase from baseline to treatment with no significant change for all groups.

3.4: Scanning electron microscope (SEM)

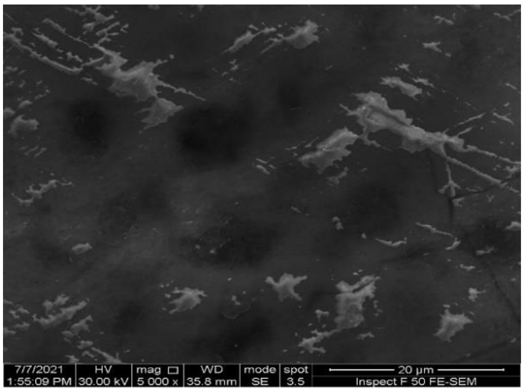
Immediately after surface treatment, typical micrographs of the G1, G2, G3, G4, G5, G6, G7 and G8 groups are shown in figure (3.3). Because of themelting caused by the laser irradiation, the enamel crystals appear to have re-solidified after melting, with no cracks or carbonization on the enamel surface.



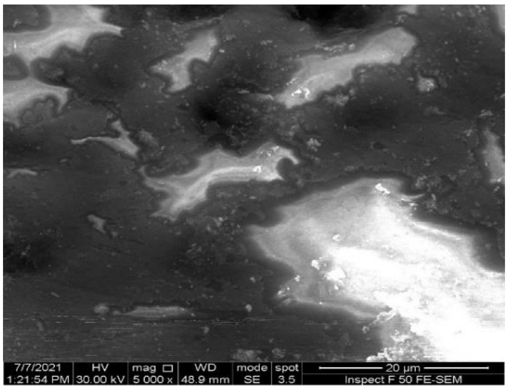
G1



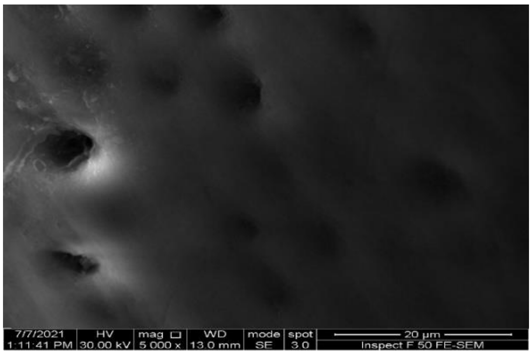
G2



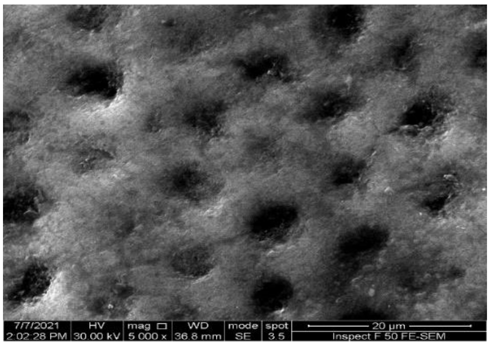
G3



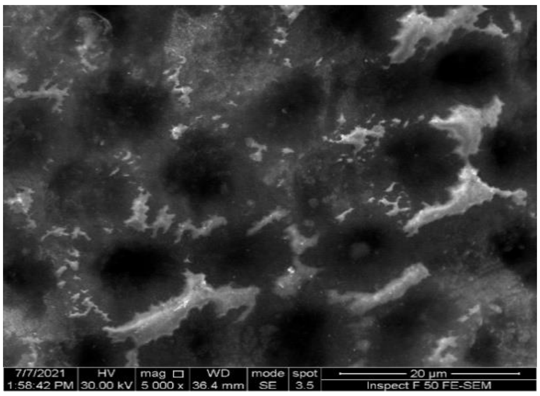
G4



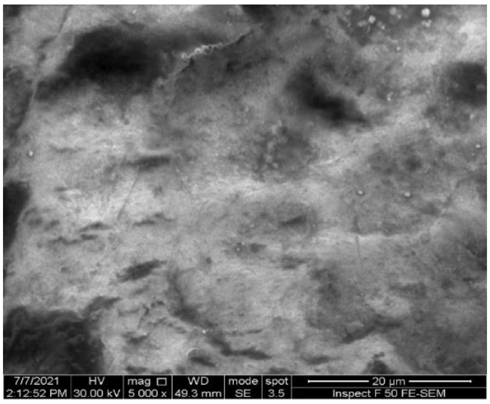
G5



G6



G7



G8

Figure (3.3): Scanning electron microscope for all groups.

3.5 Energy dispersive spectroscopy (EDS) analysis

The data obtained by EDS analysis for all groups regarding the weight percentage calcium (Ca) / phosphorous (P) ratio were shown in table (3.10) and figure (3.4). For Ca / P ratio, the weight percentage was slightly increase after laser treatment then reduced after PH cycling stage.

Table (3.10): ca/p ratio

Groups	Before laser	After laser	After PH Cycling
G1	1.694 %	--	0.063%
G2	1.795 %	1.693%	0.591 %
G3	1.629%	1.797%	0.381%
G4	1.632%	1.827%	0.437%
G5	1.667%	1.830%	0.389%
G6	1.964%	2.036%	0.710%
G7	1.516%	2.321%	0.914%
G8	2.012%	2.332%	0.792%

Results of Table (3.10) show after treatment and after demineralization, the Ca /P ratio find to be higher in G7 and G8 respectively followed by G6 while the lowest is in the control group G1.

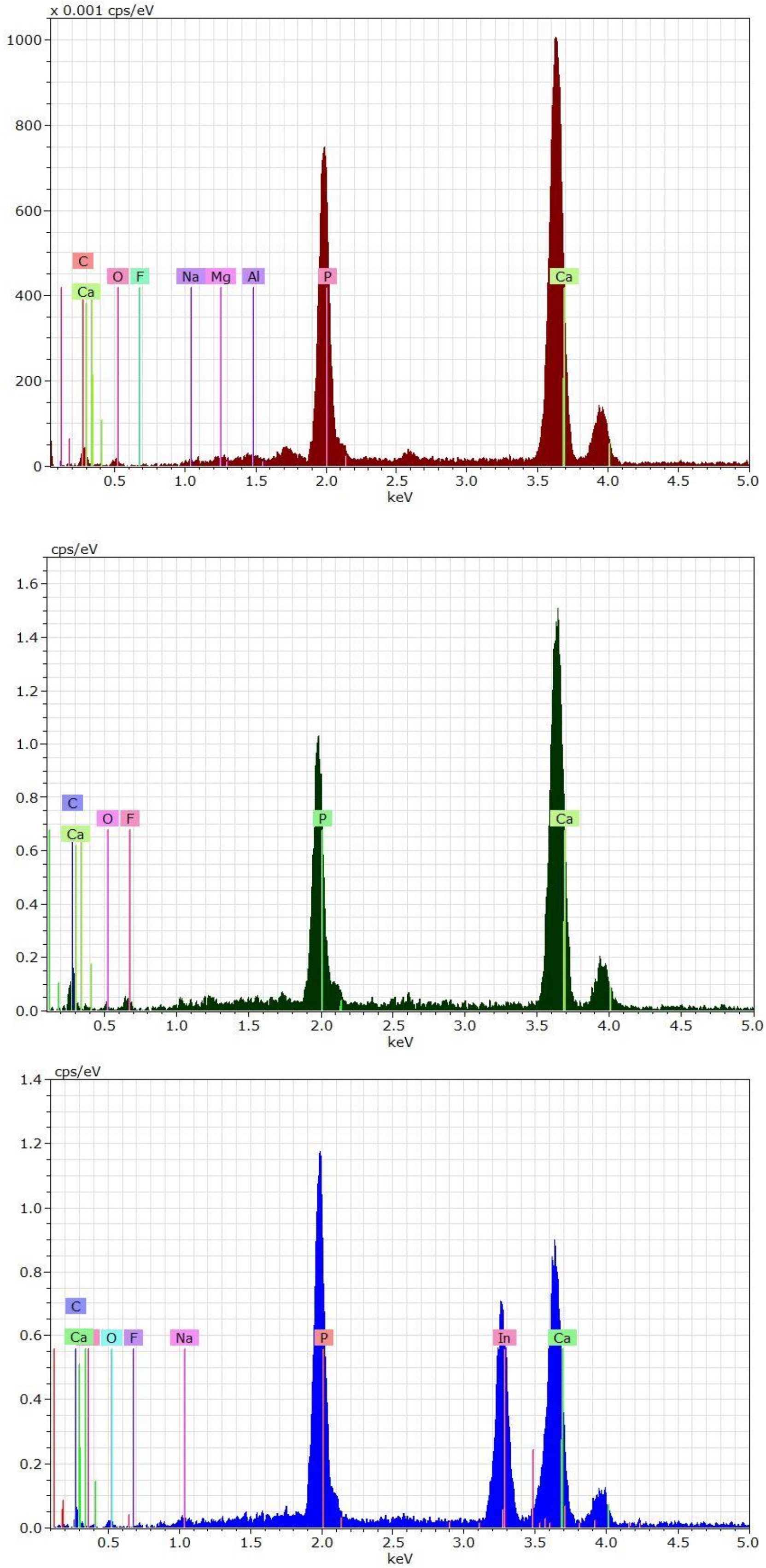


Figure (3.4): Energy dispersive spectroscopy (EDS) analysis

3.6 Discussion

The goal of this study was to investigate the impact of Er.Cr:YSGG laser or Fractional CO_2 laser with or without Acidulated phosphate fluoride on enamel acid resistance. Teeth sample consisted of 80 samples of maxillary first premolars and mandibular third molar which were extracted from Iraqi patients in Baghdad city and divided into groups to facilitate conduction of the study.

Dental caries is a chronic disease caused by a variety of factors, one of which is the formation of a cariogenic biofilm on tooth enamel. The bacteria in the biofilm produce acids, which lower the pH of the enamel. The enamel is demineralized if the biofilm is not eliminated from the dental tissue, and in more severe situations, a cavity occurs in the hard dental tissue (Nogueira et al., 2017).

As a result, in this study, for initiation of carious lesion, pH cycling procedure was followed. This method of pH cycling is simple, reliable, provide standardization and very successful in the initiation of dental caries, also is considered to be a better simulation of the vivo situation in which the enamel subjected to de- and re-mineralizing sequence. The initiation of carious lesion in the current study was conducted in ten days' cycle.

Although dental caries is a preventable disease, it is still common and remains a public health problem. Therefore, there is still a need to prevent dental caries and search for other methods for prevention, or new methods for boosting current preventive programs. One of the new preventive measures is the use of lasers and nanotechnology (Jeong et al., 2006, Cochrane et al., 2010).

Furthermore, the teeth in were divided into buccal and lingual halves in this investigation, This strategy reduces the chance of errors caused by the varied baseline mineral compositions of individual teeth, as proposed by Assarzadeh et al (Assarzadeh et al., 2021).

There are several methods used to measure the amount of enamel demineralization and compare its different degrees, as visual examination, quantitative light-induced fluorescence, the use of polarizing microscope, microradiography and microhardness tests are which is most important of these methods.

Microhardness testing is extensively utilized in laboratory research to measure the quantity of enamel demineralization since enamel hardness is related to mineral content and enamel demineralization is linked to mineral loss (Rafiei et al., 2020a) therefore it is used in this study as a measure to evaluate response to different method (laser and fluoride) planned to enhance resistance to dental caries. Vickers and Knoop microhardness tests are two of the most widely used procedures in dentistry. In this study Vickers hardness was used method because of the following: better for determining enamel microhardness in small places, the Vickers test requires less polishing due to its reduced sensitivity to the sample's surface condition (González-Rodríguez et al., 2011), High precision, the use of a single type of indenter for numerous materials and procedures, the ability to test a range of materials under varying forces are all advantages of the Vickers test (Marsillac et al., 2008). It was used as a hardness method for measuring the amount of enamel demineralization. There were three microhardness measurement intervals in this study: baseline, following laser irradiation, and after the PH challenge. These measurements clearly reflected the effect of fluoride and laser irradiation on tooth surface. Furthermore; Vickers micro hardness test helped to identify the effect of different laser types or parameter. Microhardness test treatment in this study was considered to reflect the effect of fluoride and laser as methods to enhance acid resistance of teeth, it also recommended by AJSJPtCoDUoB and Al Anni (DS, 2011, Al Anni, 2011).

Fluoride since decades plays an important role in modifications of tooth structure to resistance to demineralization therefore it is used in this study. They are also useful as therapeutic agents for inactivated or arrested caries lesions in non-restorative caries treatment (NRCT) (Slayton et al., 2018, Urquhart et al., 2019). APF gels are used as topical fluoride treatments in almost all dental offices because of their ease of application and their clinical effectiveness. In this study the application time of the APF gel was 4 min according manufacture instruction. Acidic gels enhance fluoride uptake and improve resistance to caries (Delbem and Cury, 2002). In the present study to evaluate the in vitro use of 1.23 % APF gel, in terms of its effect on the anticariogenic potential of fluoride as a topical fluoride application; to compared it with control and lasers group.

The result of present study indicated that significant increase in microhardness value in fluoride group (G2) between the base line and after fluoride treatment in microhardness test as show in the table (3-3). The hyper saturation of fluoride ions causes reprecipitation on hydroxyapatite, forming an intact superficial layer on the enamel surface. This could be attributed to the different theories in fluoride interaction with enamel. One is through incorporation into the hydroxyapatite crystal forming fluoridated hydroxyapatite which improved enamel hardness as well as it is less acid soluble than hydroxyapatite. The other is through the formation of a fluoride-rich layer containing calcium fluoride-like material (CaF2-like) over the tooth surface such interactions decrease enamel demineralization (Clark et al., 2014).

Lasers have been used to enhance caries resistance of enamel. Successful applications of lasers in caries prevention without pulpal insult requires that irradiation with specific laser wavelength, should be strongly absorbed by the enamel. Consequently, the absorbed energy results in heating up of surface layers of enamel, the heat drives out the weak, acid soluble carbonate phase and forms the purer hydroxyapatite (Rechmann et al., 2020).

In this study selection of lasers for caries prevention were tested in the pilot study. Each laser uses a different method, as well as different laser settings and irradiation parameters, to strengthen the resistance of tooth structure to caries. Different laser parameters were tested considering laser power (0.25,0.5, 0.75, 1.0 W), PPS 20 Hz, 10 % air and 1% water for Er.Cr:YSGG laser and different power (1 , 2 , 3, 4 W) , pulse duration 10.0 ms and distance 0.2 mm for Fractional CO_2 laser. After irradiation of each sample it was examined under light microscope to see the morphologic changes and surface texture, and a profilometer roughness measurement. Laser power 0.75 W and 1.0 W for Er.Cr:YSGG and 2, 3 and 4 W for CO_2 laser showed carbonization and increasing in roughness. The optimal parameter for laser irradiation should increase enamel hardness 0.25 W and 0.5 W for Er.Cr:YSGG and 1 W for CO_2 with no significant altering surface roughness as show in the table (3-8). Application of lasers with an increase in enamel roughness leads to a higher accumulation of biofilm, which increases the risk of caries and periodontal disease (Nogueira et al., 2017). However, results in this study revealed that the modifications caused by Er,Cr:YSGG and CO_2 lasers power on enamel hard tissue were no significant change in roughness test, which improve preventive requirement for laser application.

According to a recent study, irradiating enamel with the Er,Cr:YSGG laser at low power resulted in small cavities characteristic of micro ablation zones with crack and conical craters, as well as pointed enamel projections. Biofilm deposition and bacterial invasion may be aided by craters and fissures (Ana et al., 2012b). However, the modifications caused by Er,Cr:YSGG and CO_2 lasers power on enamel hard tissue were no craters , cracks and carbonization appearance on SEM test as show in figure (3-4), according to the findings of this study.

The 2780 nm wavelength of the erbium, chromium:yttrium-scandium-gallium-garnet laser is considered as a helpful laser for melting and re-solidification enamel without damaging the tooth pulp (Eversole and Rizoiu, 1997). The significant absorption of water and hydroxyapatite hydroxyl groups by this laser determines its ablation potential. “This type of laser should be utilized with a lower energy density than that used for melting and re-solidification to achieve caries prevention effects. Therefor 0.25 W and 0.5 W was used to irradiate enamel surface. As a result of the higher temperature of the enamel surface, only chemical changes would takes place (de Freitas et al., 2010).

High absorption of CO_2 laser by dental tissue. This is a result of the fact that the CO_2 laser produces radiation in the infrared region, which coincides closely with some of the apatite absorption bands, mainly phosphate and carbonate group absorption bands. During irradiation, chemical and morphological change can be induced in the dental enamel, this lead to changing the susceptibility of its modified mineral content to organic acids in the oral environment (Zancopé et al., 2016).

In this study, water level in the experimental groups was 1% to enhance laser effect, which good agreement with Meister et al (Meister et al., 2006), external water of the laser handpiece do not play a significant role in melting. As a result, water cooling should not be employed during irradiation for the purpose of caries prevention effaces from Er.Cr:YSGG laser. Using a low-energy laser with air cooling, enamel caries resistance can be achieved without increasing pulpal temperature” ,in good agreement by HOSSAIN et al and Asadollah et al (HOSSAIN et al., 2000, Asadollah et al., 2019).

Laser irradiation in G3 showed non-significant in hardness measurement over fluoride group, same result was seen when other laser group were compared to fluoride group (G4, G5). Table (3-3) indicates that laser treatment in the suggested parameter and types were equal in effect on microhardness to fluoride, therefore recommendation of laser using as caries preventive technique to exclude the application of chemical (F) and reduce the chance for exposure to

Fluoride with its complication as toxicity and fluorosis as well as all the precaution needed in fluoride application is not essential in laser irradiation, also repeated fluoride application to enhance caries resistance of the tooth is not needed. Further investigation of repeated laser application in comparison to fluoride is suggested.

For the group G4 and G5 treated with laser alone, result revealed that the mean values of microhardness increased with a statistically significant differences as compared to the base line as show in table (3-3), this may be attributed to the fact that Er.Cr:YSGG and CO_2 lasers wave length is compatible with the absorption peak of hydroxyapatite crystal which is the major component of dental enamel and then the strongly absorbed and efficiently converted to heat without damaging to the underlying or surrounding tissues causing ultrastructural modifications (melting and re-crystallization or re-solidification processes) on the irradiated enamel surface and increase microhardness (Corrêa-Afonso et al., 2012).

These findings are in good agreement with (Bachmann et al., 2008, de Oliveira et al., 2017, da Silva et al., 2019, Ana et al., 2012b, Ulusoy et al., 2020b); regarding microhardness increase but with differences in the power of laser used. The result didn't go with that of (YOSHIKAWA, 1991) and this was most probably due to different parameters involved in the lasing process as power, pulse frequency and duration of irradiation.

In this study, the combining irradiation with Er.Cr:YSGG or CO_2 lasers and the application of fluoride (G7and G8) have found that this combination results in highly significant in microhardness of enamel than any treatment that uses a laser (G3,G4,G5) or fluoride alone (G2) as show in the table (3-3). Laser irradiation causes chemical and morphological changes at the surface. More specifically, the chemical changes occur as a result of the removal of carbonated apatite, whereas morphological changes result from the increase in the surface temperature. These changes increase fluoride uptake at the surface of the tooth after the application of fluoride gel, increasing the protection of the enamel (Chin-Ying et al., 2004). Ana et al. noted an increase in the formation of calcium fluoride-like material on the enamel surface after exposing the tooth to laser irradiation prior to fluoride application, with non-significant difference between (G7 and G8) after treatment.

The mechanism of laser effect in caries or acid resistance is unfortunately still unknown but there are a variety of explanations have been given for this process.

For preventing dental caries, the melting and re-solidification effects of the lasers irradiation should promote chemical changes in dental enamel that decrease its solubility. There are some theories that explain the mechanism of the increase of enamel’s acid resistance induced by laser irradiation (Assarzadeh et al., 2021): (1) the decrease of enamel permeability due to melting and recrystallization of enamel surface ; (2) the decrease in enamel’s solubility due to the formation of less soluble substances, such as tetra calcium diphosphate monoxide; (3) the decrease in enamel’s solubility due to changes in its ultra-structure, as the reduction of water and carbonate contents, the increase in the hydroxyl ion contents, formation of pyrophosphates, and the decomposition of proteins (Bevilácqua et al., 2008, Rafiei et al., 2020b).

Carbonate loss is linked to enhanced enamel resistance to acid attacks, according to research (Bahrololoomi et al., 2015, Nair et al., 2016). Carbonate has a lower adaptability to enamel crystals, therefore after carbonate loss, the crystals are reoriented in a more stable and resistant shape. The fact that laser irradiation is absorbed by particular specific components and the radiation energy is directly transformed to heat can be used to explain this conclusion (Corrêa-Afonso et al., 2012). This would result in structural and chemical crystallographic alterations in the enamel, as well as increased acid resistance

(Apel et al., 2005). In this study, Er.Cr:YSGG laser irradiation and fractional CO_2 groups Laser produced highly significant different increased in the enamel hardness compared with the base line, this in good agreement with.(Ulusoy et al., 2020a, Ana et al., 2012a and Zancopé et al., 2016).

A statistically highly significant reduction was found in microhardness of enamel surface after pH cycling for all groups as an indication of enamel demineralization and initiation of carious lesion because any reduction in pH of the surrounding environment below critical pH (5.5) as show in the table (3-6), would create an acidic medium that causes outward movement of the minerals of tooth mainly calcium and phosphorous leaving behind micro pores and reducing hardness, so in good agreement with Yu et al (Yu et al., 2014). But when this reduction is compared to the control group, some of the experimental group still recorded acceptable hardness value.

In this study, the fluoride-treated group (G2) did not demonstrate a superior ability to prevent the progression of enamel demineralization after the PH cycling in comparison with laser groups, as show in the table (3-5). Despite the fact that highly concentrated and acidic fluoridated formulations, such as APF gel, can predispose to the formation of more CaF2-like deposits on tooth substrates. A study was suggesting that its protection in highly acidic environments may be short-lived, necessitating frequent application of the agent, and this good agreement with (Huysmans et al., 2014). On the other hand, multiple applications of fluoride gel might result in tooth discoloration, allergy or irritation, toxic effects, and time waste due to multiple clinical appointments, with no documented result. Therefore, laser irradiation may help to overcome these shortages.

The effects of fluoride on preventing tooth demineralization have been demonstrated to be improved with laser irradiation. In the table (3-5), the combination of laser irradiation with fluoride (G7and G8) had a protective effect, greatly lowering surface loss with significant different when compared to the control group. According to the results of this study, there was highly significant increase in the surface microhardness of the enamel following the combined laser irradiation and fluoride application with mean highly significant differences when compared to that in the control group, this finding is in good agreement with (Geraldo-Martins et al., 2014). The improvement of the microhardness could be attributed to increase the formation of calcium fluoride-like material on the enamel surface, which could explain why this combination treatment was able to control the progression of enamel demineralization in the current study as proposed by (Ana et al., 2012b).

The sequence of laser and fluoride application is essential in microhardness of enamel improvement , in this study the fluoride was applied after laser application because according to AlShamrani et al (AlShamrani et al., 2021), application of fluoride before laser could create mechanical barriers which reduce energy applied at the surface of enamel and prevent laser effect or alteration to enamel surface. However, in this study the combining of Er,Cr:YSGG or CO_2 laser irradiation with fluoride application reduces enamel demineralization more effectively than either laser (G3,G4and G5) or fluoride alone (G2), and this good agreement with (de Freitas et al., 2010). In this study, the combination of fluoride with this laser with parameter (G7 and G8) produced promising results with non-significant different between combination laser groups. The result indicated considerable changes with highly significant reduction in enamel surface loss, which prompted the researcher to investigate the anti-erosive properties of this laser in enamel. This finding is in good agreement with previously reported results (Zancopé et al., 2016, Ana et al., 2012a, Tagliaferro et al., 2007, Esteves-Oliveira et al., 2009, Klein et al., 2005).

This could be explained by the chemical and morphological changes induced by laser irradiation on the surface. Chemical reaction takes place when carbonated apatite is extracted, whereas morphological changes take place as the surface temperature rises. These changes occur after the application of fluoride gel, enhancing fluoride uptake at the tooth's surface and improving enamel protection (Ana et al., 2012a). Also laser irradiation could be changes in the treated enamel structure where the rods of the laser-treated part are overlapping, sharper, and more interconnected with each other. This explains the high microhardness and resistance of the treated tooth against demineralization and in good agreement with (Hamoudi et al., 2020).

EDS analysis revealed changes in the concentrations of enamel components. Calcium and phosphorus existing in hydroxyapatite crystals are the main inorganic ingredients of the dental hard tissue (Dilber et al., 2013). The treated samples demonstrated increase in the calcium to phosphorus ratio which refers to the re-distribution of mineral components during melting and re-solidification (Hamoudi et al., 2020) with the appearance of fluoride in combination and fluoride groups.

In the table (3-9), the relationship between enamel microhardness and mineral content, based on weight measurements, was demonstrated. The enamel microhardness increased with the increase of the Ca/P ratio, and this enhances tooth resistance to decay. Basically, the higher the Ca/P ratio is the lower solubility the calcium phosphate compound. The control group had a lower Ca/P molar ratio compared to other groups in this study after PH cycling. Mineral and chemical changes taking place in the enamel at laser energy are responsible for the decrease in permeability and acid penetration within the internal layers. Laser energy modifies the Ca/P mineral ratio and forms steadier and lesser acid soluble compositions, consequently lowering the chances for acids to attack the tooth and cause decay. This could affect solubility, permeability, or adhesive specifications of the dental enamel (Hamoudi et al., 2020). It has been demonstrated that enamel mineral concentration reduction could increase porosity, leading to an increased caries susceptibility and lower microhardness of dental enamel (Akkus et al., 2017).

In this study, a demineralization-remineralization cycling was suggested by AL-Bazzaz (Al-Bazzaz, 2017) in which 6 h per day the sample were exposed to acid at 4.5 and repeated for 8 days. Naturally in the oral cavity the possibility of exposure to the acid at PH 4.5 for continuously rare. when compared to the clinical condition the ph cycling is different. Due to the different in the biological parameters such as saliva flow rate, content, buffering capacity and duration of acid exposure. In this study a high benefit for laser were achieved as there was a high reduction in the microhardness due to ph cycling which might be different from the clinical condition. As a result of this difference we might get a superior benefit from laser in the clinical condition.

3.7 Conclusion

1. Er.Cr:YSGG or fractional CO_2 lasers irradiation with suitable power settings has a positive effect on enamel tooth surface and increasing enamel microhardness without cracks or carbonization.
2. Er.Cr:YSGG or fractional CO_2 lasers irradiation enhance microhardness equal to fluoride application when the power was 0.5 W for Er.Cr:YSGG and 1.0 W for Fractional CO_2 .
3. Er.Cr:YSGG laser irradiation with 0.5 W , 20 Hz , 10 % air and 1% water and fractional CO_2 laser irradiation with 1 W, pulse duration 10.0 ms, interval 1.0 ms , distance 0.2 mm combination with Fluoride can significantly inhibit the progress of demineralization process on enamel surface without ablation or cracks was appeared in SEM micrograph.
4. The irradiation temperature was safe < 1°c at 2mm distance from the enamel surface therefore these lasers can be used safely in caries resistance.

3.8 Suggestion

1. Application of laser treatment to enhance acid resistance or caries clinically.
2. Effect of CO_2 and Er.Cr:YSGG lasers on initial caries.
3. Evaluate lasers effect on caries causative bacteria as *S.Mutans* or *Lactobacillius*.
4. Further investigation on the enamel crystallographic and chemical changes due to laser irradiation using Ramman spectroscopy and X-ray diffraction.
5. Assess and compare other types of topical fluoride agents with or without lasers.
6. Effect of CO_2 or Er.Cr:YSGG lasers on primary teeth.

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الاستنتاجات: عند مقارنتها بمجموعة التحكم، تطبيق فلوريد الفوسفات الحمضي باستخدام تشعيع ليزر Er.Cr:YSGG بمقدار 0.5 واط ، 20 هرتز ، 1٪ ماء ، و 10٪ تشعيع بليزر الهواء أو ثاني أكسيد الكربون عند 1 واط من الصلادة المحسنة لسطح المينا وتجنب إزالة المعادن من المينا .

تعزيز الصلابة الدقيقة لمينا الأسنان باستخدام ليزر Er.Cr :YSGG + CO2 مع أو بدون تطبيق الفلورايد

الخلاصة

الخلفية: ينتج عن تشعيع الأسنان بالليزر تفاعل بين الضوء والمكونات البيولوجية لمادة الأسنان الصلبة. تأثير الليزر هو سبب التغيرات الهيكلية والكيميائية المينا. قد يؤدي العلاج المشترك لعامل الفلوريد الموضعي مع الليزر إلى زيادة صلابة المينا وتقليل تنقية المينا.

الأهداف: - الغرض من هذه الدراسة هو مقارنة فعالية استخدام ليزر Er.Cr :YSGG، CO2 مع أو بدون فلوريد الفوسفات الحمضي (APF) لتحسين مقاومة حمض الأسنان. (النظر في الخشونة والصلابة الدقيقة وتحليل العناصر)

المواد والطرق: - تم الحصول على ثمانين من أسنان المينا من الضواحك الأولى والثالثة السفلية تم تقسيمها عشوائيًا إلى ثماني مجموعات (ن = 10): :(G1) مجموعة التحكم (بدون معالجة) ، :(G2) تم تطبيق فلوريد الفوسفات الحمضي (APF) مع القطن لمدة 4 دقائق , (G3): Er.Cr:YSGG ليزر عند 0.25 واط ، 20 هرتز ، 1٪ ماء و 10٪ هواء ، (G4): Er.Cr:YSGG ليزر عند 0.50 واط ، 20 هرتز ، 1٪ ماء و 10٪ هواء ؛ G5: ليزر ثاني أكسيد الكربون عند 1.0 واط ، طاقة 10 مللي جول ، مدة الوقت 10.0 مللي ثانية ، فاصل 1.0 مللي ثانية ، مسافة 0.2 مللي متر ؛ (G6): Er.Cr:YSGG ليزر عند 0.25 واط ، 20 هرتز ، 1٪ ماء و 10٪ هواء وفلوريد الفوسفات الحمضي ؛ (G7): Er.Cr:YSGG ليزر عند 0.5 واط ، 20 هرتز ، 1٪ ماء و 10٪ هواء وفلوريد الفوسفات الحمضي ؛ (G8): ثاني أكسيد الكربون عند 1.0 واط ، الطاقة 10 مللي جول ، المدة الزمنية 10.0 مللي ثانية ، الفاصل 1.0 مللي ثانية ، المسافة 0.2 ملم وفلوريد الفوسفات الحمضي. تم إخضاع العينات لدورة الأس الهيدروجيني لمدة 10 أيام بعد معالجتها. تم اختبار العينات لصلابة ديجيتال فيكرز الدقيقة على (9.8 نيوتن) على سطح المينا على خط الأساس ، بعد التشعيع بالليزر وبعد دورة الأس الهيدروجيني. كما تم قياس خشونة السطح قبل وبعد العلاج. تم تقييم عينات مختارة من جميع المجموعات عن طريق مسح مجموعة المجهر الإلكتروني بعد تشعيع الليزر. تم اختيار عينات من جميع المجموعات لاختبار نسبة الكالسيوم / الفوسفور باستخدام التحليل الطيفي المشتت للطاقة (EDS) عند خط الأساس ، بعد المعالجة وبعد دورة درجة الحموضة PH. تم تحليل التاريخ إحصائيًا باستخدام اختبارات ANOVA و Tukey HSD عند ($\alpha = 0.05$).

النتائج: كان هناك فروق ذات دلالة إحصائية في قيم الصلابة الدقيقة بين خط الأساس وبعد العلاج في مجموعات الفلورايد والليزر والفلورايد + الليزر ($P < 0.05$) أظهرت زيادة معنوية في قيم الصلابة الدقيقة. ومع ذلك ، بالمقارنة مع خط الأساس ، وجدت مجموعات الفلورايد والليزر و (الليزر والفلورايد) بعد الصلادة الدقيقة لدورة PH أعلى في مجموعات الفلورايد + الليزر بينما أقلها في المجموعة الضابطة (G1)مع اختلاف كبير. اختلاف غير معنوي في قيمة الخشونة بين خط الأساس وبعد المعالجة. أظهر SEM أنه لا توجد علامة على الكربنة أو الشقوق في مجموعات الليزر مع بعض التعديلات السطحية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
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تعزيز الصلابة الدقيقة لمينا الأسنان باستخدام ليزر Er.Cr :YSGG + CO_2 مع أو بدون تطبيق الفلورايد

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