Ministry of Higher Education and Scientific Research
University of Baghdad
Institute of Laser for Postgraduate Studies

Bactericidal Effect of 940nm Laser on Enterococcus faecalis in Endodontic Treatment (in Vitro Study)

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

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2020 A.D **1442 A.H**

بسم اللهِ الرَّحْمَنِ الرَّحِيمِ

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صدق اللَّهُ العظيم

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Dedication

I dedicate this work to my family, thanks for your unconditioned support.

Mustafa

Acknowledgment

First, I would like to thank **Allah Almighty** for supporting me to finish this work.

I wish to express my admiration and respect to Prof. **Dr. Hussain A. Jawad**, **Dean** of Institute of Laser for Postgraduate Studies for his full support and kind attention to all the students.

I would like to express my appreciation to my supervisor **Dr. Salah Abdul Mahdy**, Consultant Maxillofacial Surgeon-Ministry of Health, I was very lucky to be under his supervision.

Many thanks to Head of Department **Assist Prof. Dr. Bassima M. Ali** for her scientific advises and follow up during the work.

Also I would like to thank **Dr. Mahmood Shaker Mahmood**, the associate dean of the institute of Laser for post graduate studies for his follow up and kindness.

I would like to thank **Dr. Mohammed Ghaleb** (Institute of Laser for Postgraduate Studies / Baghdad University) for his valuable assistance and guidance during the work and the statistical analysis.

Thanks to **Al-Mamoon specialized dental center** for their corporation with me in bacterial sampling.

Special thanks to **Dr.Ahmed Mohsen** (G.B dentist) for his support and cooperation during the study.

I would like to express my grateful to **Al-Sibd center for research and scholarly published SRP** for their assistance in my article publishing.

Thanks to **Salah Sabah Mohsin** (MSc microbiology student) for his cooperation with me. I am grateful for his assistance.

Thanks to **Dr. Noor Ali Alwardi** (MSc laser dentistry) for her advises and support.
Also I like to thank **Assist Prof. Dr**. **Ghada Mohammed** (College of Science, University of Baghdad) for her instructions and follow up during the study.

Mustafa

Abstract

Background: the main reason for endodontic treatment failure is the presence of some species of bacteria inside the root canal system such as *enterococcus faecalis*. Those microorganisms are more resistant to the disinfection agents causing persistent intra radicular or extra radicular infection. **Objective:** there are two purposes of this invitro study; the first one is to test the antimicrobial activity of 940nm diode laser on *E.faecalis* biofilm within root canals and the second one is to estimate that if the use of diode laser could enhance the activity of NaOCl. **Materials and methods:** fresh bacterial samples were collected from infected root canals of patient undergo root canal treatment and *E.faecalis* species was identified by using VITEK analyzer. Then 40 single rooted teeth were prepared and inoculated with the bacterial culture and incubated for two weeks anaerobically. After that all teeth specimens were divided into four groups each one has ten samples. Group A had received no treatment as it considered control group, group B was disinfected by diode laser only, group C was disinfected with 5.25% NaOCl and group D treated with 5.25% NaOCl together with laser radiation. Swabs were taken by introducing paper point #F3 into each canal then placed in Luria-Bertani broth and incubated for 24 hours then drop of the broth streaked on blood agar media and the number of Colony forming units were recorded manually. **Results:** the 940nm diode laser showed the weakest antibacterial agent as it decreased the bacterial load in group B to about 26.3% as compared to group A while 5.25% sodium hypochlorite exhibited good antibacterial effect on E.faecalis as there was 71.9% bacterial reduction in group C. The best bactericidal effect achieved when NaOCl exposed to laser radiation during the disinfection procedure as they

decreased the bacterial growth to 95.5% (in comparison with group A). **Conclusion:** the 940nm diode laser is best used as co-adjunctive with other root canal chemical irrigants during disinfection procedure. When used alone it gives weak bactericidal effect.

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List of abbreviations

Abbreviation	Term
Etc	and so forth
Nd;YAG	Neodymium doped Yttrium –Aluminum Garnet
CO2	Carbon dioxide
CEJ	Cemento-enamel junction
PH	Potential of hydrogen
P.micra	*Parvimonas micra*
P.alactolyticus	*Pseudoramibacter alactolyticus*
F.nucleatum	*Fusobacterium nucleatum*
F.faecalis	*Enterococcus faecalis*
NiTi	Nickle-Titanium
NaOCl	Sodium hypochlorite
EDTA	Ethylenediamine tetra acetic acid
RCT	Root canal treatment
CHX	Chlorohexidine gluconate
EM	Electromagnetic
He-Ne	Helium-Neon
IR	Infrared
PDT	Photodynamic therapy
cm	centimeter
W	Watt (power unit)
LLLT	Low level laser therapy
J	Joul (energy unit)
ATP	Adenosine Triphosphate
ºC	Degree Celsius (unit of temperature)

Sec	second
UV	Ultra violet
F	femtosecond
nm	nanometer
Er,Cr:YSGG	Erbium chromium-doped yttrium scandium gallium garnet.
LAI	Laser assisted irrigation.
UTI	Urinary tract infection.
Min	minute
Ml	Milliliter.
#10	Size 10 (refers to file size)
CFU	Colony Forming Unit
No	number
CFU/ml	Colony Forming Unit per milliliter
Mm	micrometer
CW	Continuous emission mode
LB	Luria-Bertani broth.
S	significant
NS	Non significant
HS	Highly significant
P	Probability
SE	Standard error
SD	Standard deviation

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Chapter One

Introduction and Basic Concepts

1.1 Introduction

The Root canal is a term refers to the tract inside the root of the tooth which extend from the canal orifice coronally to the apical foramen apically and contains the pulp tissue. Root canal curvature varies from almost straight to severely curved canal following in that the root curvature[1].

While the pulp is a soft connective tissue lies in the pulp chamber and the root canal. It originates from the mesoderm and contains blood vessels and sensory fibers and has nutritive, formative and sensory functions (sense of heat, cold drilling, etc..)[2]. Figure 1.1 shows the anatomy of the tooth.

The term pulpitis defined as inflammatory response of the pulp tissue occurs primarily as a result of bacterial invasion or its by-products to the pulp, other causes of pulpitis may be chemical irritation (such as that accompanying adhesive restorations) or heat elevation(as in case of excessive tooth polishing). The obvious symptom of this condition is pain, treatment options for this case ranging from caries elimination, root canal treatment, or even tooth elimination[3].

Endodontic therapy is a general term encompasses multiple procedures aiming at resolving the inflamatory condition of the pulp or creating bacteria free environment of the root canal[4,5].

In dentistry lasers used extensively, they include diode, erbium family, Nd:YAG, and CO2. They emit radiation ranging from 810 nm to 10600 nm. Many researches made on its ability in cleaning and disinfecting root canals and showed that it enhance smear layer removal and bacterial disinfection[5].

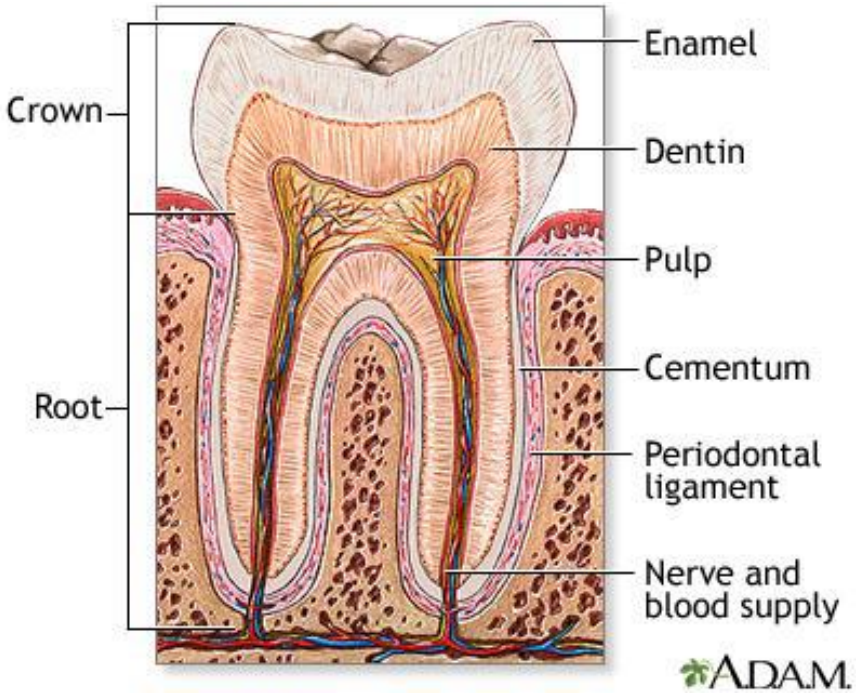


Fig (1.1) tooth anatomy and its composition[6]

1.2 Dental caries

Defined as a chronic, preventable, treatable multifactorial disease resulting in devastation and demineralization of hard tissue of the teeth. It occurs as a result of sugar fermentation produced by sugar fermenting bacteria. There are four major factors in caries formation which are: time, bacteria, fermentable sugar, and susceptible tooth surface.[7]

dental caries affecting 80-90% of people in the world making it one of the most wide spread chronic diseases.[8]

Microbiologically, *Streptococcus mutans* and *Lactobacilli* are strongly responsible for caries initiation, as *S.mutans* ferments sugars such as sucrose producing lactic acid and ATP. lactate accumulation shown to be responsible for the so called "local acidification" within the plaque environment on the tooth surface.[9] periodic acidification in turn upsets pH

equilibrium leading to shifting in demeneralization-remeneralization balanse toward minerals loss and consequently caries formation.[10]

1.2.1 Infected root canal

when the tooth infected by deep carious lesion or get fractured bacteria from the oral environment may reach the pulp tissue and consequently will colonize root canals and in this situation normal root canal will become infected root canal. Bacterial biofilms which defined as microbial community co-aggregated within self-produced extracellular matrix providing environmental protection against immune system, antimicrobial agents, and other local stresses, these biofilms could developed inside the root canal and if not treated by successful endodontic treatment may lead to periapical lesion.[11]

1.2.1.1 Consequences of root canal infection

Bacterial invasion to the root canal system result in pulp infection which in turn leads to pulp necrosis. If the infection within the root canal persists without treatment a apical lesion will developed.[12]

Pulp necrosis if happened without bacterial invasion may not necessarily leading to apical periodontitis. Nevertheless pulp necrosis caused by microorganisms could lead to apical periodontitis as the root canal is no longer bacteria free along with the absence of the immune system.[13]

Apical periodontitis is an inflammatory response from the periodontium against bacteria contaminating the root canal. The continuous immune reaction with the bacterial toxins result in creation of apical bony lesion.[14]

The type of bacterial species found within the infected canal can to a large extent influence the composition and virulence of total microbiota, and with such influence apical lesions may persist.[5]

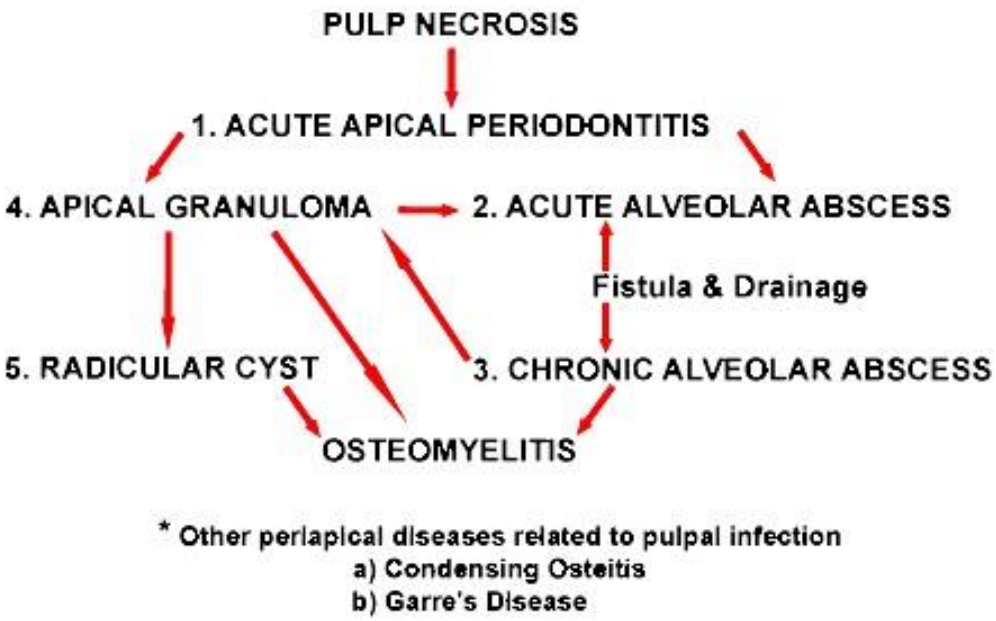


fig. (1.2) The possible consequences of root canal diseases.[15]

Root canal infection can be categorized according to the time in which bacteria invade the root canals as follow[5]:

1- ***primary infection*** (virgin infection); in which microorganisms firstly enter and coloniz the pulp tissue.

2- ***secondary infection***; occurs as a result of microorganisms that were not present during the primary infection and invade the root canal system during or after endodontic treatment.

3- ***persistent infection****;* it is caused by microorganisms that were present from the primary or secondary infections and resist the biomechanical instrumentation and disinfection and were able to withstand long terms of nutritional deprivement after root canal treatment.

Bacterial diversity and evenness differ in the forementioned categories, and for more detailed information they will be explained in the next section.

1.2.2 Types of bacteria in infected root canal[5]

In primary root canal infection:

Gram-negative bacteria include:

Fusobacterium, Dialister, Porphyromonas, Prevotella, Tannerella, Treponema, Pyramidobacter, Campylobacter,and Veillonella.

Gram-positive bacteria

Parvimonas, Filifactor, Pseudoramibacter, Streptococcus, Propionibacterium, Olsenella, Actinomyces, Peptostreptococcus, Enterococcus and Eubacterium).

In secondary and persistent infection

Gram positive bacteria

Streptococci, *P. micra*, *Propionibacterium* Species, *P. alactolyticus*, *Actinomyces* species, lactobacilli, *E. faecalis*, *Olsenella uli*

Gram negative bacteria

Prevotella species, *F. nucleatum*, *Campylobacter rectus*[5]

figure (1.3) illustrat the prevalence of each bacterial species in persistent infection.

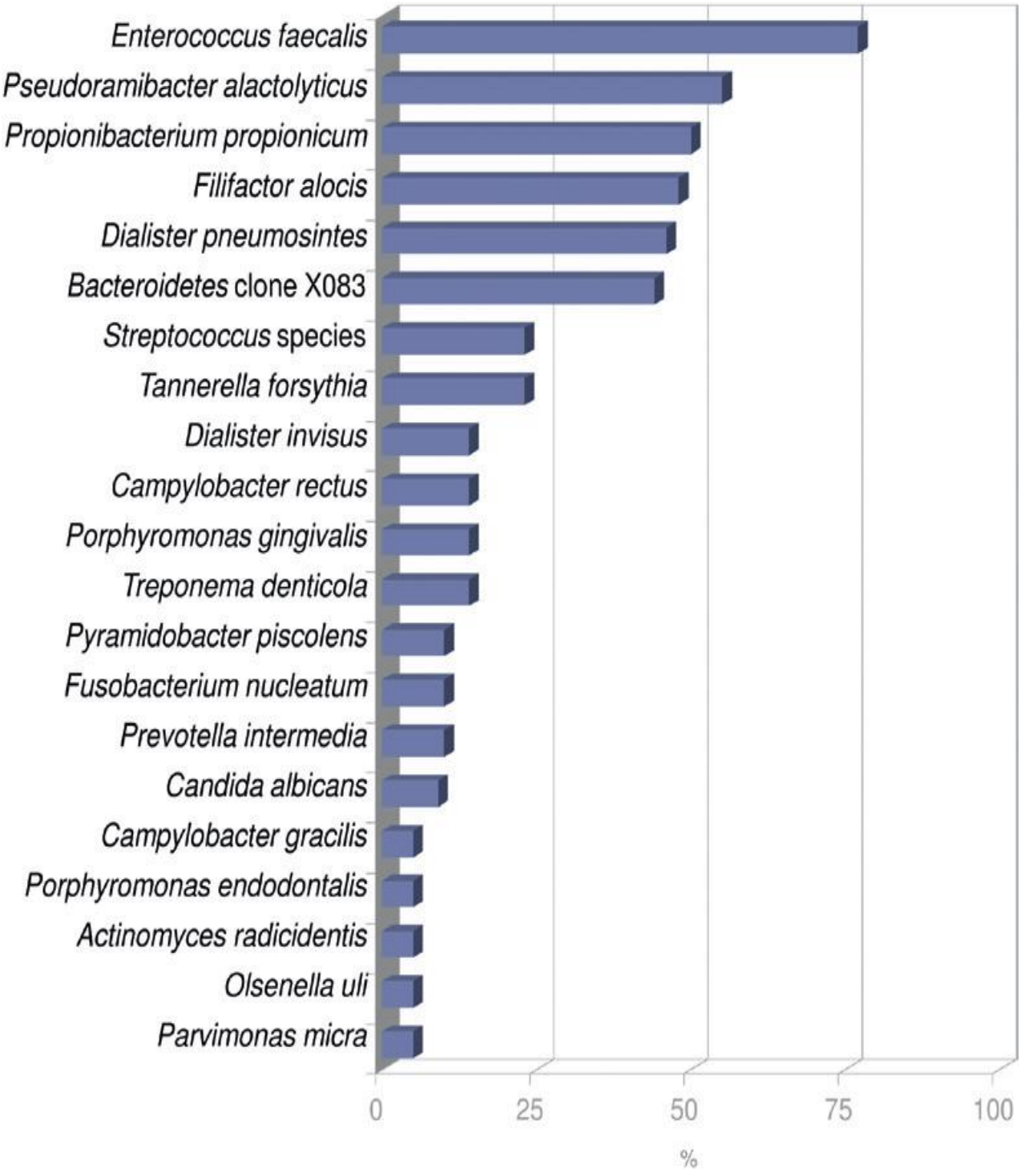


Fig. (1.3) Bacterial prevalence in endodontically treated teeth with persistent infection.[5]

As seen in the previous figure and in many studies that *Enterococcus faecalis* is the most abundant bacteria collected from reinfected root canals, this is related to the fact that this bacterium has the ability to resists the

antimicrobial agents (e.g. 5% sodium hypochlorite), forming biofilm, withstand long term starvation period, invading the dentinal tubules, and due to the active proton pumping in the cell membrane this bacteria can resists high pH environment and consequently resists calcium hydroxide intracanal medicament.[5,16,17]

1-2-3 Treatment of infected root canal

Root canal treatment may be finished in single or multiple visits depending on the type of infection. Before any intervention a radiograph should be taken to the infected tooth in order to know how many roots does it have and their morphology. Also, any signs of apical lesion will be seen in the radiograph. After that the tooth must be anaesthetized by giving local anesthesia injection. In cases of pulp necrosis, anesthesia may not be needed, however, it is preferably to give local anesthesia for more patient comfort.[18]

A rubber dam will be applied around the tooth to isolate it from saliva and contamination. Then access cavity will be made in order to to remove necrotic pulp tissue and other debris from the pulp chamber and root canal. Shaping and cleaning of root canals obtained by using stainless steel or NiTi instruments called endodontic files. They are organized according to the size from the smallest one to the largest and used according to that sequence along the full length of the canal together with root canal irrigant.[5] A temporary filling should be placed after completing the cleaning and shaping step in order to keep the canal isolated from oral environment and preventing debris entrance inside the canal. An intracanal medicament will be left inside the canal in case of infection to improve lesion healing.[19]

After shaping and cleaning, the root canal will be obturated with a rubber thermoplastic material called gutta-percha accompanied by endodontic sealer. Finally, the tooth will be restored with a permanent restoration.[19,20]

1.2.4 Root canal irrigants

Irrigant solutions are essential for successful root canal treatment. These solutions enhance the mechanical debridement ,done by files, by flushing out necrotic pulp, dentin chips and disinfect microorganisms. Also they provide lubrication for endodontic files thus enhancing their action.[21] the most frequently irrigant solutions used are:

A) Sodium Hypochlorite (NaOCl)

This antibacterial agent prepared in various concentrations ranging between (0.5%-8%). It acts by destroying the phospholipid of the cell wall of bacteria making it a strong antimicrobial root canal irrigant. Also, it has the ability to dissolve organic matter of the pulp and dentin. The main disadvantage of sodium hypochlorite is its cytotoxicity to the periapical area in case of solution escaping out of apex. **Zandi** and his workers showed that there is no considerable difference in bactericidal effect of NaOCl for different concentrations and by increasing the concentration its toxicity will be increased too.[22,23]

B) EDTA (Ethylene Diamine Tetra Acetic acid)

A chelating agent widely used in RCT has low or no antibacterial effect but enhancing the action of other irrigants by removing the smear layer formed during mechanical instrumentation by forming a stable complex with Ca ions in dentin. It is available in concentration range of (15%-17%).[24]

C) Chlorohexidine gluconate (CHX 12%)

One of the most powerful bactericidal root canal irrigants. it shown to be biocompatible with the native tissue. CHX has proposed to replace sodium hypochlorite during instrumentation and disinfection but its shortage is that it lacks organic matter dissolution so it cannot dissolve pulp tissue.[25,26]

Now days, laser technology is widely used in endodontic treatment. Because of its high power and unique light properties it could be used during cleaning and disinfection in combination with root canal irrigants. laser shown to be effective in bacterial reduction and smear layer removal and recently it is used to activate irrigation solution by producing mechanical disturbances.[27]

1.3 Basics of laser

Laser is an acronym for (light amplification by stimulated emission of radiation). It is a machine that emits light at specific wavelength by stimulating atoms or molecules to emit radiation then amplifying it to produce coherent beam of radiation. Laser emission falls within narrow range of electromagnetic spectrum ranging between ultra violet, visible, and infrared wavelengths.[28] as seen in the figure below

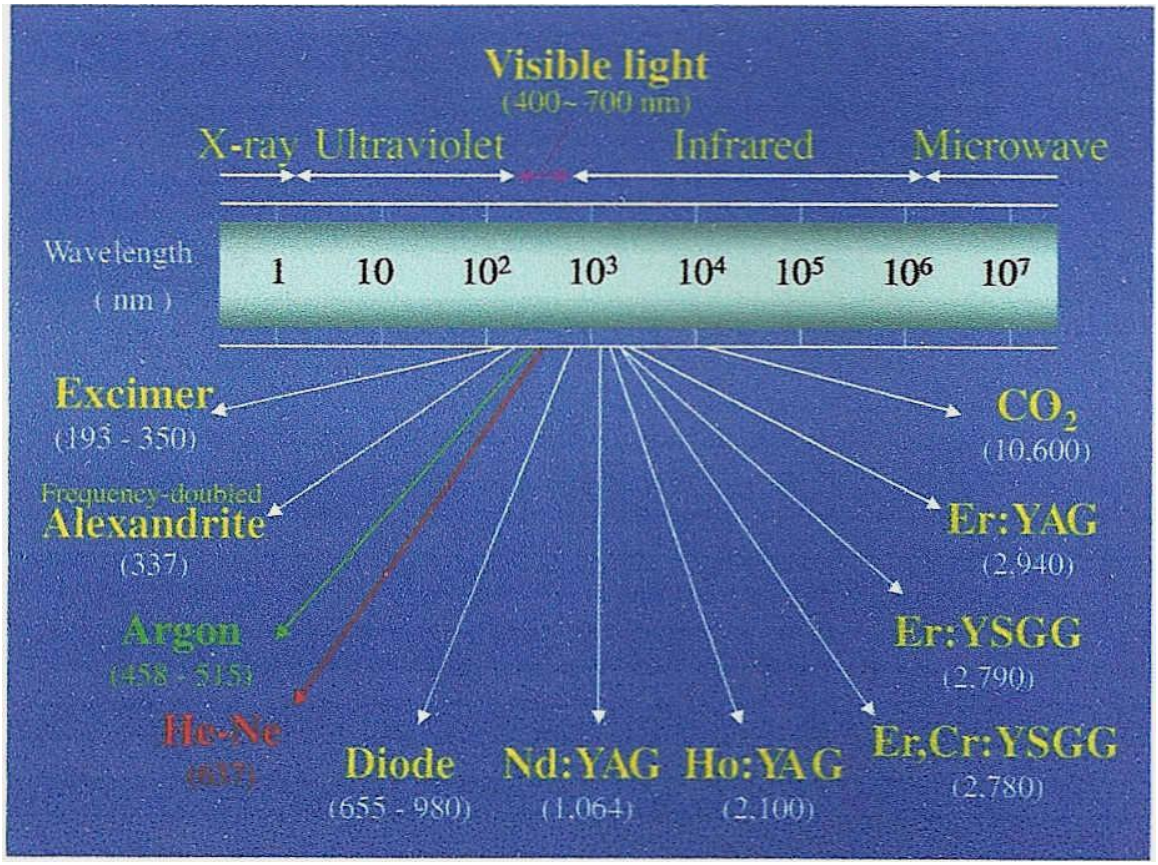


Fig. (1.4) Laser wavelengths in EM spectrum.[29]

1.3.1 History of laser[28,30]

In 1916 Albert Einstein suggested that under optimium conditions atoms may release superflowing 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 emmited 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 CO2 and Nd:YAG lasers were constructed.

1.3.2 Population inversion

in physics, to produce laser emission, a redistribution for atomic energy levels must occur in the atomic system. Under normal conditions, in temperature equilibrium a system of atoms has more atoms in lower energy levels than those in higher energy levels. The distribution of atoms (population) in the different energy levels remain constant during the absorption and emission processes. So by pumping energy into the atomic system a population inversion will occur by which more atomes will be in higher energy levels than in the lower energy states.[31]

1.3.3 components of laser system

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.[32]

B) Reflecting mirrors: At the two ends of the laser cavity there are two parallel mirrors or two polished surfaces (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 resonators* by reflecting the radiation back and forth 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.[33,34]

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 energy states 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.[33,35,36]

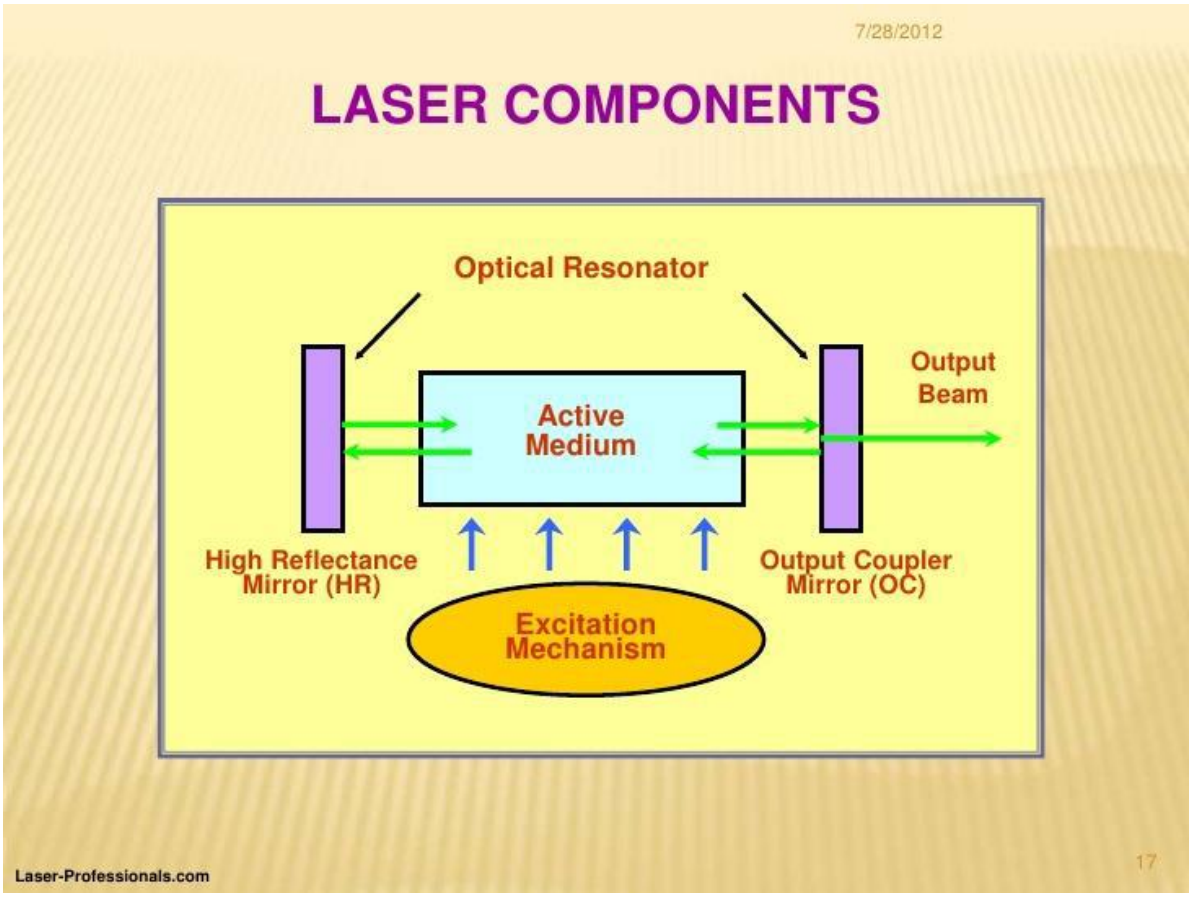


Fig. (1.5) Major parts of laser unit.[37]

1.3.4 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.[38]

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.[38,39]

C) Coherence: ordinary light is not coherent as it originates from various atoms. Coherence occurs as a result of the stimulated emission (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.[40]

D) 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. Collimation property in laser gives the advantage that laser beam can be focused to a very small area with high intensity apart of ordinary light which lacks this property.[41]

1.3.5 Laser effects on biological tissue

When laser irradiate tissue there are four effects may appear[42]:

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:** when the angle of incidence less than the refraction angle a total reflection of the incident beam occurs. In case of true reflection, the angle of incidence will equal to the angle of reflection. If the interface is inhomogeneous or rough some scatter may occur.
3. **Transmission:** the laser beam does not undergo any interaction with the medium during its passage through it, then it will emerg out unchanged or partially refracted.
4. **Scattering:** it occurs as a result of poor interaction between laser beam and tissue resulting in insufficient attenuation to the incident beam completely. The beam rays move in uncontrolled directions and its energy will decreased with distance and some distortion will occure. When laser beam of short wavelength hits the tissue back scattering may result.

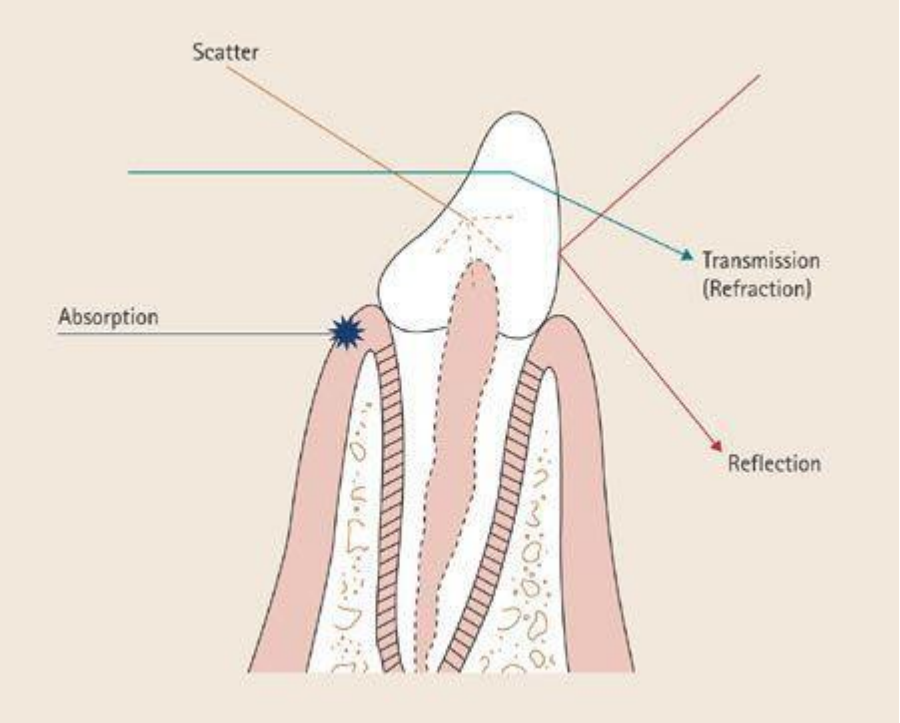


Fig. (1.6) Effects of laser on tissue[42]

There are several factors that govern laser light absorption by the target tissue[43]:

- Exposure time
- Laser wavelength
- Angle of incidence of laser beam
- Composition of target tissue
- Contact vs non contact modes
- Thickness of tissue
- Wetness of surface

1.3.6 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[44]

Various interaction mechanisms result when laser energy hits biological tissue, these are categorized as follow:

1.3.6.1Wavelength 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 very low power densities (usually 1 w/cm) with long exposure times ranging from seconds to continuous exposure.[45]

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.[33]

1. photodynamic therapy

As mentioned above PDT based on photochemical reaction and need the presence of three elements: laser power, photosensitizer, and molecular oxygen[46]. 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[47].

2. Biostimulation

Also known as Low level laser therapy (LLLT) is a treatment modality that utilizes light of single wavelength to stimulate or inhibit a specific biological function. LLLT doesn't elevate tissue temperature, instead it produce the effect though photobiostimulation within the target tissue[48]. 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 adenosin triphosphate (ATP) production which in turn facilitates oxidative and other cellular process and result in enhancing healing, vascularization, growth along with other mechanisms[49].

Elson and Foran studied (in 2015) 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[50].

II. Thermal interactions

Photothermal interactions achieved when laser energy transformed into heat inside tissue. This is typically occur at power densities between 1-10^6 w/cm^2 with exposure time ranging between 1msec-100msec. 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[33,47]. Fig 1.7 Illustrates the different thermal effects on tissue.

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.

Fig.(1.7) The most important photothermal effects on tissue when absorbing laser radiation[51].

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[45,52].

1.3.6.2 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 known to be very clean and well defined without causing any mechanical or thermal damage[45,53].

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 vary high peak powes. 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[45,54].

1.4 Laser in dentistry

According to the absorption by tissue chromophore, lasers divided into hard tissue lasers and soft tissue lasers. (Fig. 1.8 and table 1.2)

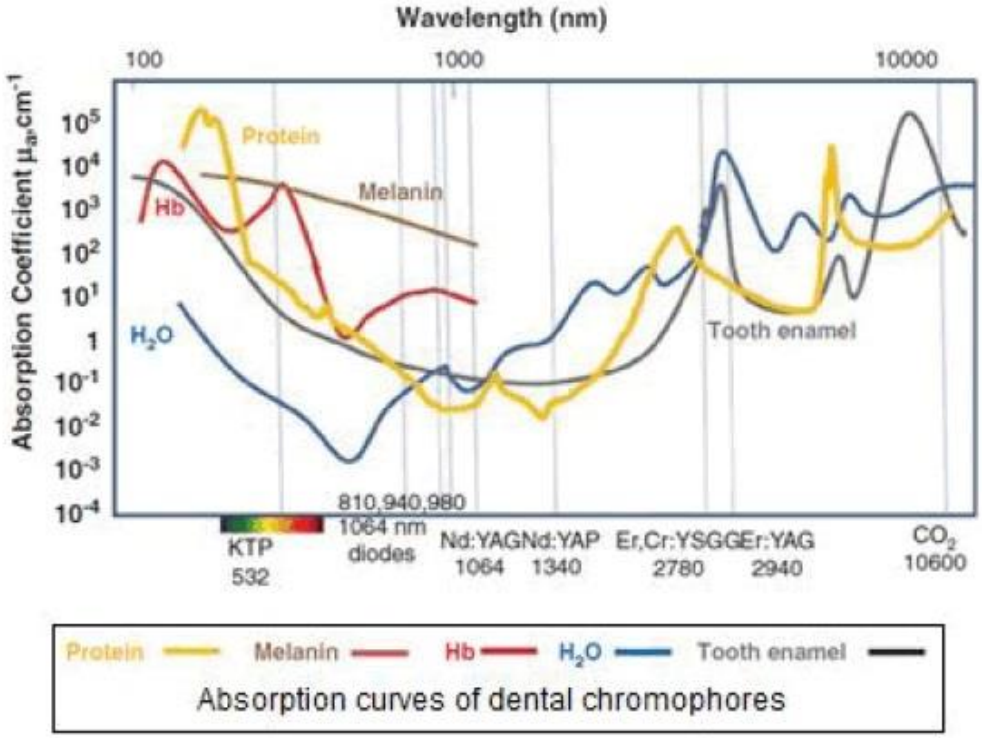


Figure (1.8) : Absorption coefficient of different tissue chromophores in relation to laser wavelength[55] .

Laser name &wavelength (nm)	Target chromophore	Emission mode	Dental use
Argon (488nm,514nm)[56]	Red colored pigment (hemoglobin and melanin) camphoroquinon	Continuous	Composite polymerization, Soft tissue surgery, bleaching
Diode[57] **810nm,940nm, 980nm**	Hemoglopin melanin	Continuous, Gated pulse	Bleaching Periodontal procedures Endodontic disinfection
Nd:YAG 1064[58]	Hemoglobin And melanin	Free running pulsed	Soft tissue treatment(non surgical sulcular depridement, endodontic disinfection)
Er,Cr:YSGG 2780nm[59]	water	Free running pulsed	Hard and soft tissue surgeries (limited action on hard tissue)
Er:YAG 2940nm[59]			
Carbon dioxide 10600 nm[60]	water	Continuous	Soft tissue surgery, provide excellent hemostasis

Table 1.1 the mostly used dental laser with their target chromophores and dental use.

1.5 Bactericidal effect of 940nm diode laser

Treatment of enamel and dentin by laser undergoes continuous development, new wavelength, new treatment procedures are implied in dentistry. In endodontic treatment, low level laser therapy (biostimulation) and high intensity laser are used as adjunctive to the conventional root canal treatment to improve the outcome of the treatment[61].

Multiple wavelengths of high-power lasers were studied in previous researches regarding root canal treatment and demonstrated the thermal effect of laser in disinfection procedures of dentinal tissue, both in decreasing the bacterial count in infected root canal and in apical surgery (apicectomy) at the site of surgery. The major advantage of laser is its sterilization capability over other conventional root canal bactericidal agents[62].

Diode 940 nm laser is widely used in dentistry due to its low cost, portability, small size, and its wide applications in oral treatments. The photothermal effect of 940nm wavelength shown bactericidal effect especially in contaminated root canal as it destroys bacterial cell wall[63].

The 940nm wavelength is poorly absorbed by water and hydroxyapatite as compared with Er,Cr:YSGG so its penetration depth is long (>1000 micrometer) inside dentin, by this mechanism it will cause photothermal disruptive effect for microorganisms in unreachable areas (inside the tubular system) that could not be reached by conventional root canal irrigants[63].

Because of structural variations between Gram-positive and Gram-negative bacteria, the Gram-negative bacteria are more easily to destroy by this laser[63].

In root canal treatment, a number of methods are utilized to activate the irrigation solutions. Studies showed that heating up NaOCl to body temperature could enhance the bactericidal effect of this solution. The studies revealed that the continuous agitation of the irrigant solution (sodium hypochlorite) increases tissue dissolution activity[64].

Sonic, ultrasonic tips, and hand agitation all are methods used to agitate root canal irrigants. authors suggested that laser beam is capable of agitate irrigant solution in a process known as laser activated irrigation(LAI) and some authors revealed that the agitation by laser is more powerful than the conventional methods and has better effect in smear layer removal[65].

1.6 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[66]:

- 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: involve lasers wavelengths in the visible region (400-700 nm), they don not cause skin or eye damage if viewed in less than quarter second (<0.25s).
- 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 reflacted 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 visibile 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.

1.7 Literature review of root canal disinfection

In the teeth with pulp necrosis the spaces of the pulp cavity become unprotected by the immune system and this makes this environment favorable to the proliferation of various microorganisms[67]. Such microorganisms form biofilms on the walls of the root canals and colonize the adjacent dentin structure[68] and are associated with the formation and persistence of periapical lesions[69,70]. Clinically, from a therapeutic point of view, an important focus is oriented to achieve negative microbiological culture prior to root canal[71] and the efficacy of antisepsis protocols can be routinely assessed through the microbiological culture and confocal laser scanning microscopy[68].

In 1936 Walker first suggested the use of sodium hypochlorite in root canal therapy, and in 1941 Grossman demonstrated the tissue-dissolving ability of chlorinated soda when used in double strength. Spangberg in 1973 said that 0.5% of NaOCl has good germicidal activity,

he reported that the greater the contact time, the more effective the NaOCl irrigant is. This is especially important in necrotic cases where 5.25% of NaOCl used for 40 minutes was found to be effective.[72]

The most recent development in endodontic treatment is the use of lasers. Since the development of the ruby laser by Maiman in 1960 and the application of the laser for endodontics by Weichman in 1971, a variety of papers on potential applications for lasers in endodontics have been published[73].

The first laser use in endodontics was reported by Weichman& Johnson (1971) who attempted to seal the apical foramen invitro by means of a high power-infrared (CO2) laser. Subsequently, attempts were made to seal the apical foramen using the Nd:YAG laser[74].

Bactericidal effect of laser is attained by causing changes in bacterial cell wall. Because of the complex three layer membrane, gram negative bacteria are very sensitive to irradiation, and only very small densities of energy result in severe damage to the cell membrane of bacteria[73].

An indirect irradiation with 1W causes obvious changes to the cell membrane of bacteria. A number of large, vesicle formations of different sizes can be observed (so called membrane blebbing) which covers the bacteria totally or partly. The blebbing phenomenon is the result of the inner layer of the membrane splitting from the two outer layers. This change of the cell membrane impacts upon the barrier function and since the cell coat is also the site of a most diverse enzyme system; one can also assume that a slight restructuring of the membrane disturbs the cell's metabolism substantially. The cell wall of the gram positive E. faecalis shows an astonishingly

high resistance against the laser irradiation. Low energies (1W) show almost no changes to these problematic bacteria. With the application of multiple irradiations, visible damage of the bacteria can be detected, but there can still be a few unaltered cells.[75]

1.8 The aims of the study

1- to evaluate the antibacterial efficacy of a diode 940nm laser on the *enterococcus faecalis* biofilm in infected root canal system.

2- to compare the antibacterial efficacy of the diode 940nm laser and 5.25% NaOCl irrigating solution separately and when combined inside root canal

Chapter Two

Materials and Methods

2.1 Materials

No.	Name of material	Company & origin
1	Metal k files	Dentsply, Switzerland.
2	Eppendorf tubes	China.
3	Test tubes	China.
4	Luria-Bertani broth	Himedia, India
5	Test tube rack	China.
6	UTI agar plates	Oxoid, United Kingdom.
7	NaOCl 5.25%	Cerkamed, Poland.
8	CHX 12%	Cerkamed, Poland.
9	EDTA 17%	PD, Australia
10	Protaper files	Fanta, China.
11	Plastic syringe	Jiangsu Zhiyu, China.
13	Irrigation needles	Sinalident, China.
14	Ultrasonic tip	Wood Beeker, china
15	Sterile water	Aqua chemical, Egypt
16	Paper points	Sinalident, China.
17	Composite resin	Colten, Switzerland.
18	Temporary filling	Temp it, Spident, South Korea)
19	Micropipette	Dragonlab, China.
20	Gas burner	Piezo, usa
21	Inoculation loop	China
22	Endodontic ruler	Dentsply, switzerland

Devices

no	Device name	Company and origin
1	Diode laser	Biolase, USA
2	Thermocouple	Amprobe, USA
3	Incubator	Zhongxing, China
4	autoclave	Cryste, Thailand.
5	Vitek analyzer	Biomerieux, France
6	Microscope	Opticamicroscopes, Italy.
7	endomotor	Eighteeth, china
8	vortex	Karl Korb, Germany.
9	Anaerobic jar	Oxoid, United Kingdom.

Other materials:

Single-rooted teeth

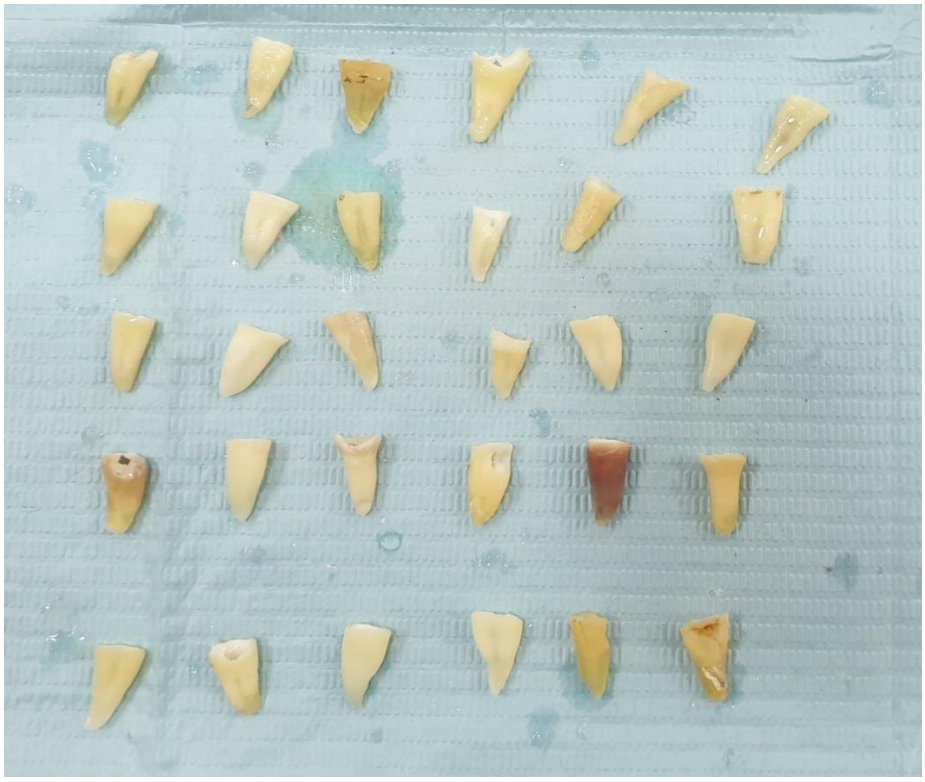


Fig. (2.1 A) some of the prepared teeth.

Fig. (2.1 B) NaOCl

Fig.(2.1C) EDTA

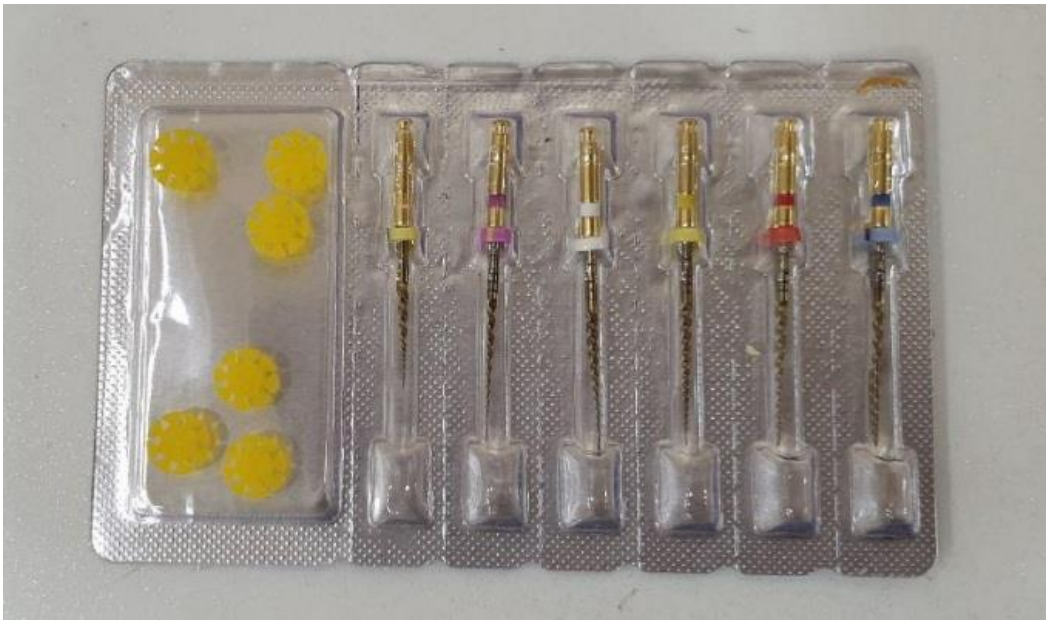


Fig.(2.1 D) Protaper files

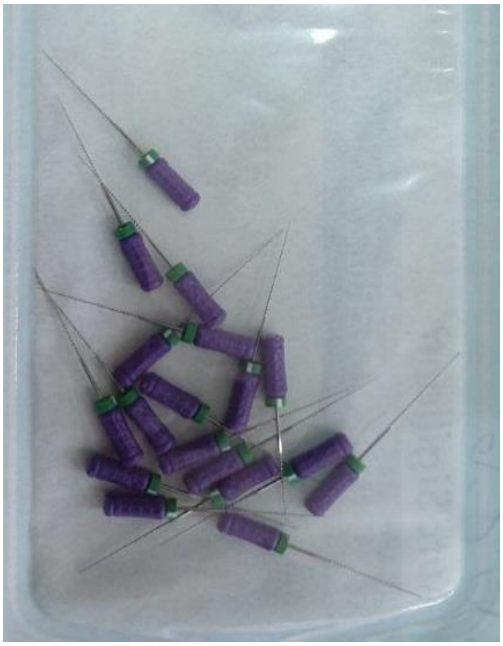


Fig.(2.1 E) Metal k files

Fig.(2.1 F) irrigation needle

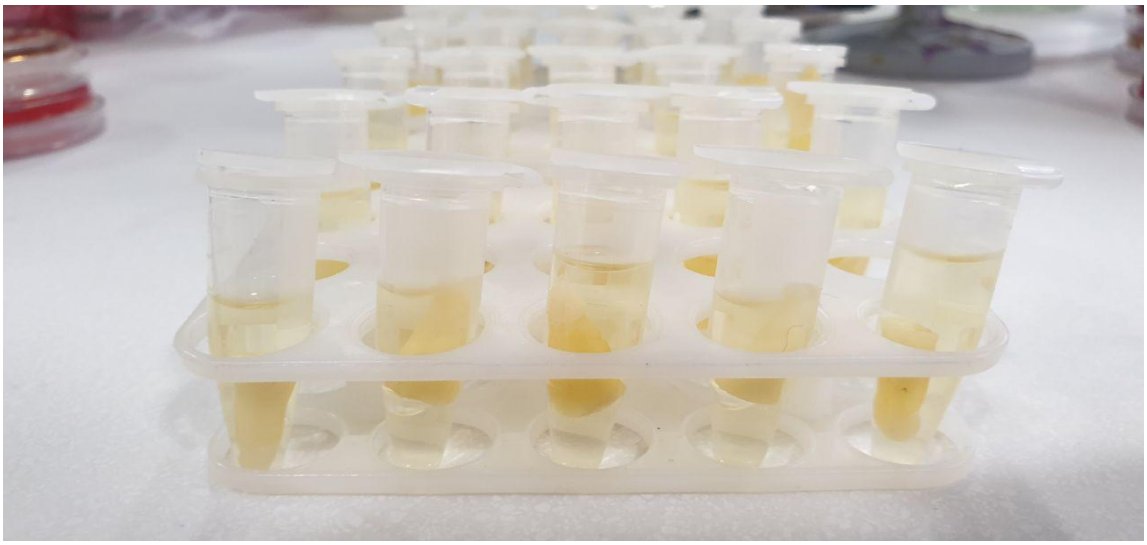


Fig.(2.1 G) Eppendorf tubes filled with broth

Fig.(2.1 H) Autoclave

Fig.(2.1 I) Micropipette

Fig.(2.1 J) Vitek analyzer

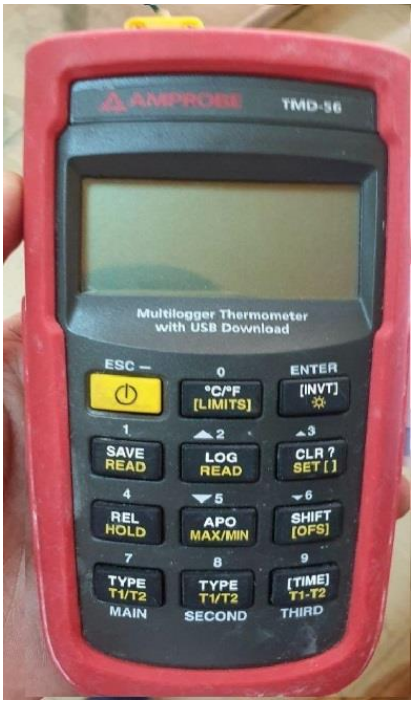


Fig.(2.1 K) Thermometer

Fig.(2.1 L) UTI agar media

Fig. (2.1M) incubator

2.2 Methods :

Many methods are used in evaluation the bactericidal effect of any antimicrobial agent. In our study, the methods used are as below:

2.2.1 *E.faecalis* isolation

In this study, swabs taken from 30 infected root canals for patients under RCT at Al Mamoon specialized dental center.

Sterile k-files size #10 were introduced inside canals with watch winding motion then streaked on UTI and nutrient Agar media and left upon the agar. Then all these media incubated at 37°C under anaerobic conditions for 48 hours.

After two days incubation, colony forming units appeared on the media. The next was gram stain.

The laboratory work done at Dar Alamal laboratory for medical analysis and it took about three months.

NOTE: most of the work done under covid-19 quarantine.

2.2.1.1Urinary tract infection media (UTI media)[76]

A chromogenic medium for the presumptive identification and differentiation of all the main micro-organisms that cause urinary tract infections (UTIs).

Composition:

1- peptone (15gm/liter)

2- chromogenic mix (13gm/liter)

3- agar (15gm/liter)

4- Final pH 7.0 ± 0.2

Description

Chromogenic UTI Medium (Clear) contains two specific chromogenic substrates which are cleaved by enzymes produced by *Enterococcus* spp., *Escherichia coli* and coliforms. In addition, it contains tryptophan which indicates tryptophan deaminase activity (TDA), indicating the presence of *Proteus* spp. It is based on Cystine Lactose Electrolyte Deficient (CLED) Medium which provides a valuable non-inhibitory diagnostic agar for plate culture of other urinary organisms, whilst preventing the swarming of *Proteus* spp.

The chromogen, X-glucoside, is targeted towards ß-glucosidase enzyme activity, and allows the specific detection of enterococci through the formation of blue colonies.

The other chromogen, Red-Galactoside, is cleaved by the enzyme ß-galactosidase which is produced by *Escherichia coli*, resulting in pink colonies.

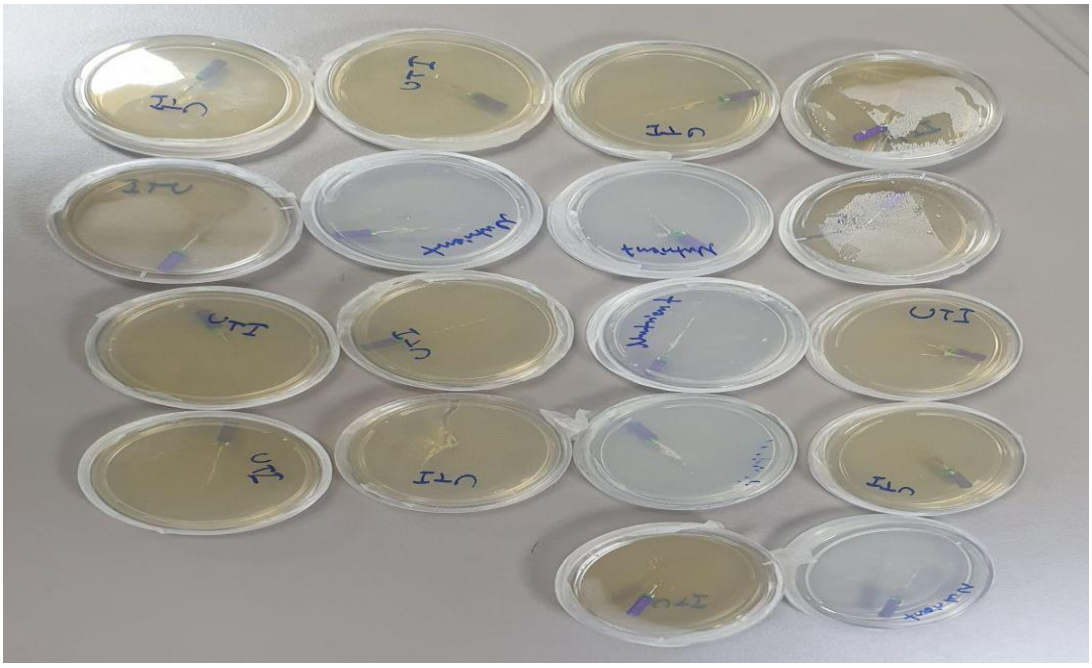


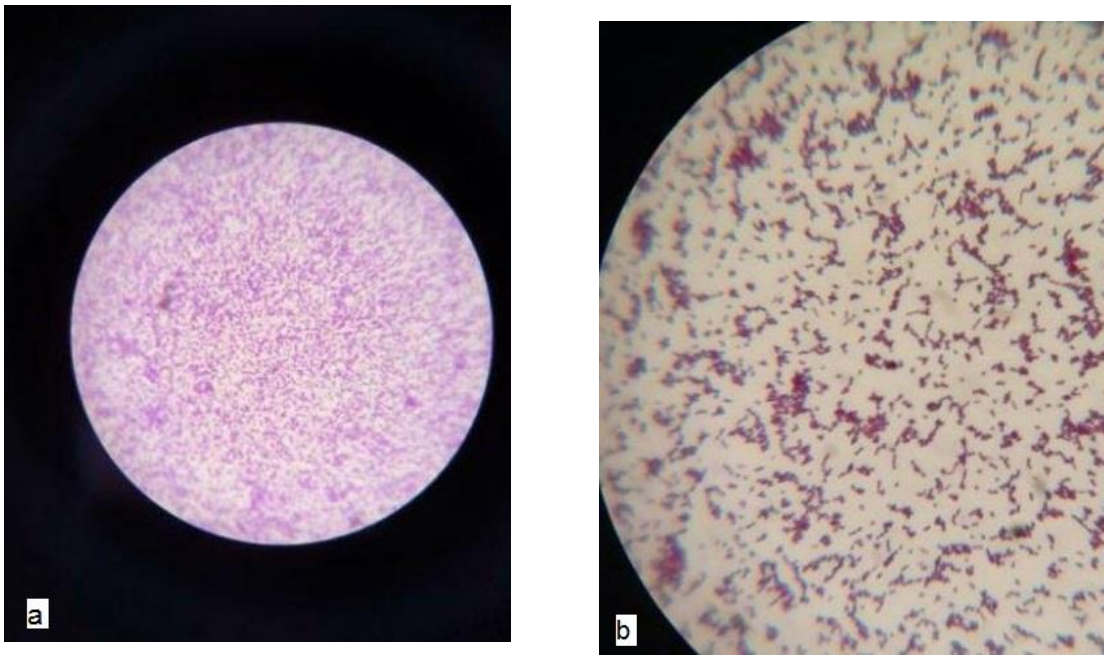
Fig. (2.2) UTI and Nutrient agar media after streaking with k files.

2.2.1.2 Gram Stain:

A biochemical test used to differentiate between Gram-negative and Gram-positive bacteria according to the basis of cell wall staining with a crystal violet and the treatment with solvent (alcohol).

As the cell wall of Gram-negative bacteria thin and more permeable so the solvent could remove the crystal violet stain and the cell will appears red. While Gram positive bacteria has thicker and less permeable cell wall that make it more resistant to the solvent and appears blue or violet[77,78].

As *Enterococcus faecalis* falls within Gram positive group so it appeared purple under microscope after staining. the figure below shows *E.faecalis* under microscope after staining.



Fg. (2.3) : Enterococcus faecalis with Gram stain under microscope (a) x10 magnificatiuon (b) x40 magnification E.faecalis appears as short chains

2.2.1.3 VITEK analyzer[79]:

After gram stain the bacteria was diagnosed by vitek analyzer for final diagnosis of *E.faecalis*.

VITEK 2 compact is a fully automated system that performs bacterial identification and antibiotic susceptibility testing. VITEK offers: Intuitive software. User interface screen for immediate notification of system status to increase productivity.

Principle: The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with

information on card type, expiration date, lot number and unique card identification number.

Test kits available include ID-GN (gram negative bacillus identification), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility) and AST-GP (gram positive susceptibility).

The Vitek 2 Antimicrobial Susceptibility Tests (AST) are for most clinically significant aerobic gram negative bacilli, Staphylococcus spp., Enterococcus spp., and Streptococcus agalactiae. Susceptibility results are available for bacteria in less than 18 hours

Pure isolates of organisms to be tested may be taken from Trypticase Soy Agar with 5% sheep blood (BAP), chocolate agar, Maconkey, and Columbia Sheep Blood Agar (CBA).

bioMérieux Customer:

Microbiology Chart Report

Printed Dec 3, 2019 07:40 CDT

Patient Name: Mustafa, .
Location:
Lab ID: Mustafa

Patient ID: Mustafa
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterococcus faecalis

Source: ..

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:**	Final
Selected Organism	93% Probability **Bionumber:**	**Enterococcus faecalis** 554012765773642		
ID Analysis Messages				

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

2.2.2 Preparation of teeth specimens

40 extracted human teeth used in this study. All teeth are permanent single rooted with single canal having approximately the same dimensions and did not undergo root canal treatment before. After debriding the root surface, all teeth specimens soaked in 5.25% sodium hypochlorite solution for 30 minutes then rinsed in normal saline till the preparation in the next day.

All teeth were sectioned from the cervical area in order to obtain uniform working length of 15 mm. each root canal patency was assessed by using K-files (Dentsply Maillefer, Switzerland) #10, #15, #20. All roots canals were prepared by using Protaper system SX-S1-S2-F1-F2-F3 (Fanta, China) as shown in figure 2.4.

Fig. (2.4) Root canal instrumentation

After each file irrigation was performed by injecting 3 ml of 5.25% NaOCl using a 30-gauge needle (Sinalident, China). The irrigation needles were placed 2 mm short from the apex in each canal. The total irrigation time for each canal was 10 min/canal.

After instrumentation with the final file, all canals were irrigated with 2ml of 17% EDTA (EDTA 17%, PD, Australia) for 3 minutes followed by a final rinse with 5.25% sodium hypochlorite for 3 minutes. Both solutions were activated with an ultrasonic tip for 30 seconds.

By using sterile water, a final wash was made to all canals then the canals dried with paper points.

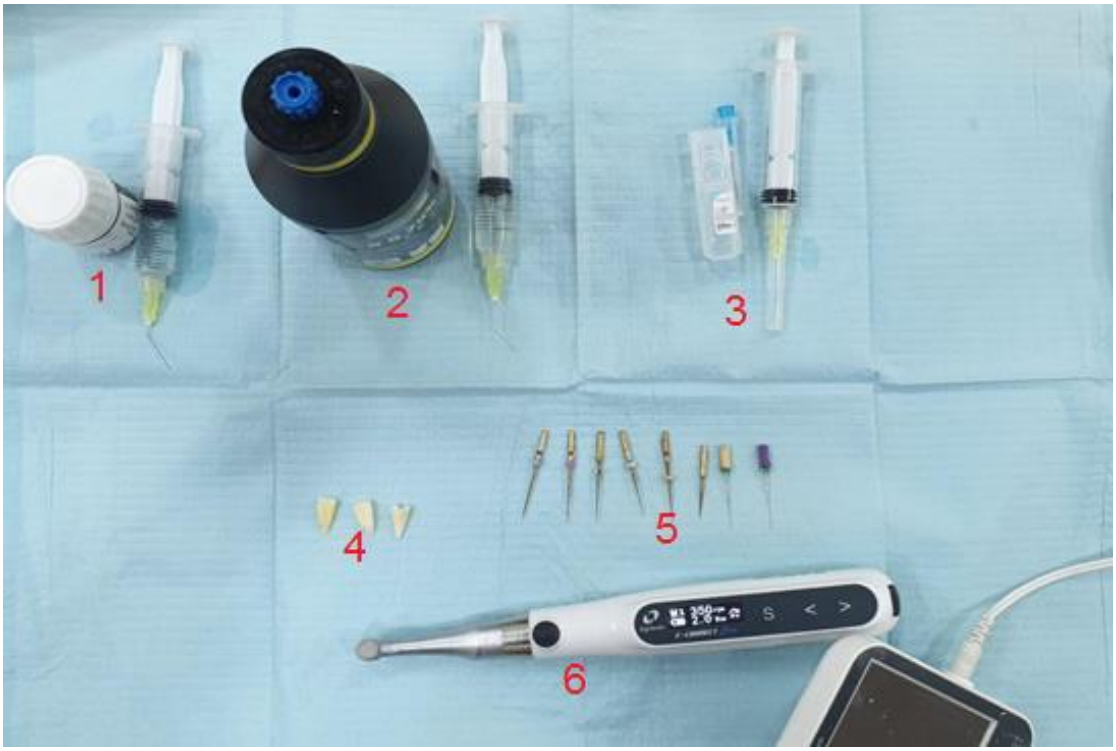


Fig. (2.5) Instruments and materials used in root canal instrumentation

1-EDTA, 2-5.25%NaOCl, 3-steril water, 4-teeth, 5-endodontic files, 6-endomotor.

After completing biomechanical instrumentation the apical foramen was sealed with composite restorative fillings (Brilliant Everglow, Coltene, Switzerland) then all the specimens autoclaved for 20 minutes at 121ºC. After sterilization, each tooth placed in eppendorf tube containing 2 ml of sterile Lysogeny broth and incubated for 48 hours at 37 °C. daily screening for the broth showed no sign of turbidity as in Figure 2.6

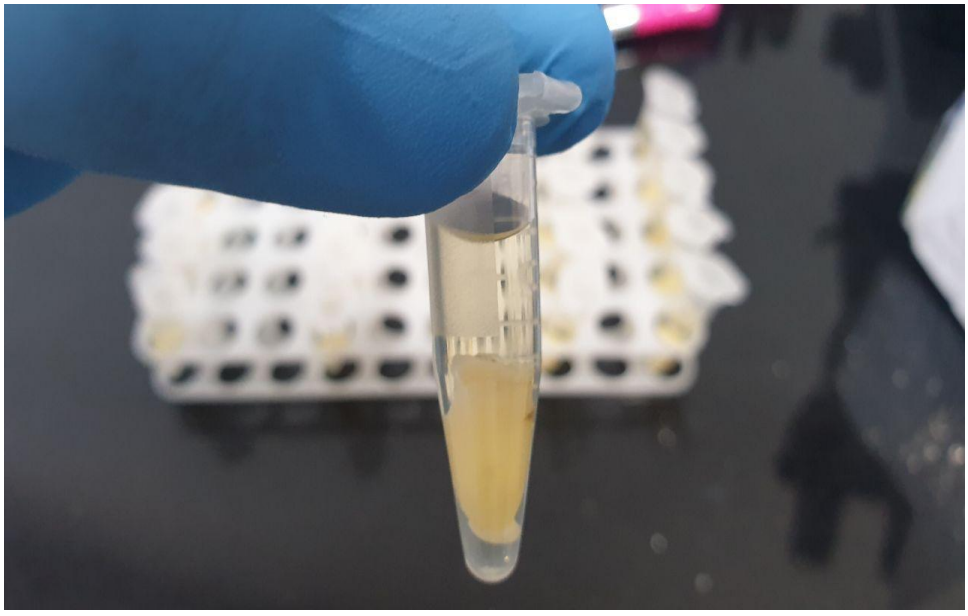


Fig. (2.6) Daily screening reveals clear broth after autoclaving.

2.2.3 Experimental root canals infection

All specimens were inoculated with an overnight culture of enterococcus faecalis in lysogen broth. The bacterial suspension matched the turbidity of McFarland 0.5 scale spectrophotometrically. In each specimen 20 µl of the suspension was inoculated by using micropipette as shown in figure 2.6, then the canals were sealed coronally with temporary fillings and incubated for two weeks at 37°C under anaerobic conditions

(by using anaerobic jar, fig2.7) into test tubes with 2 ml of lysogen broth changing it every three days.

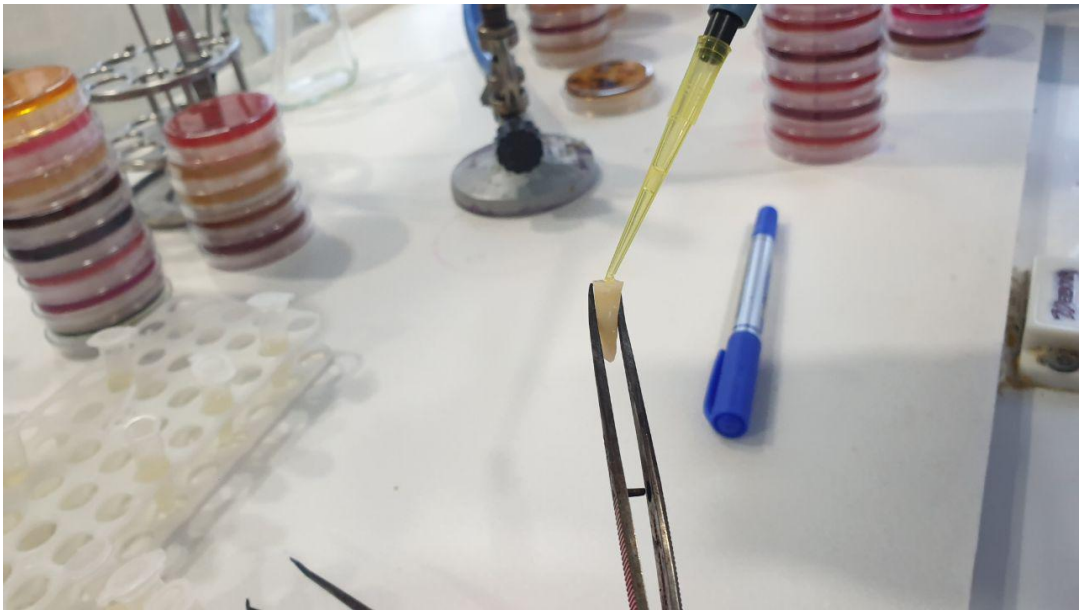


Fig.(2.7) Tooth specimen inoculation with bacteria.

Fig. (2.8) Anaerobic jar used to provide oxygen less environment for bacterial growth.

2.2.4 laser setup

The laser used in this study was Epic 10 diode laser (Biolase, USA) and it has unique specifications as follow:

1- The main parts ,figure 2.9 are :

1. Wireless footswitch
2. Base console
3. Protective goggles
4. Delivery system that consists of
    - ✓ Fiber optic which is reusable
    - ✓ Handpieces for surgical and whitening purposes
    - ✓ Disposable tips of different diameters (200, 300and 400 μm) for surgical handpiece

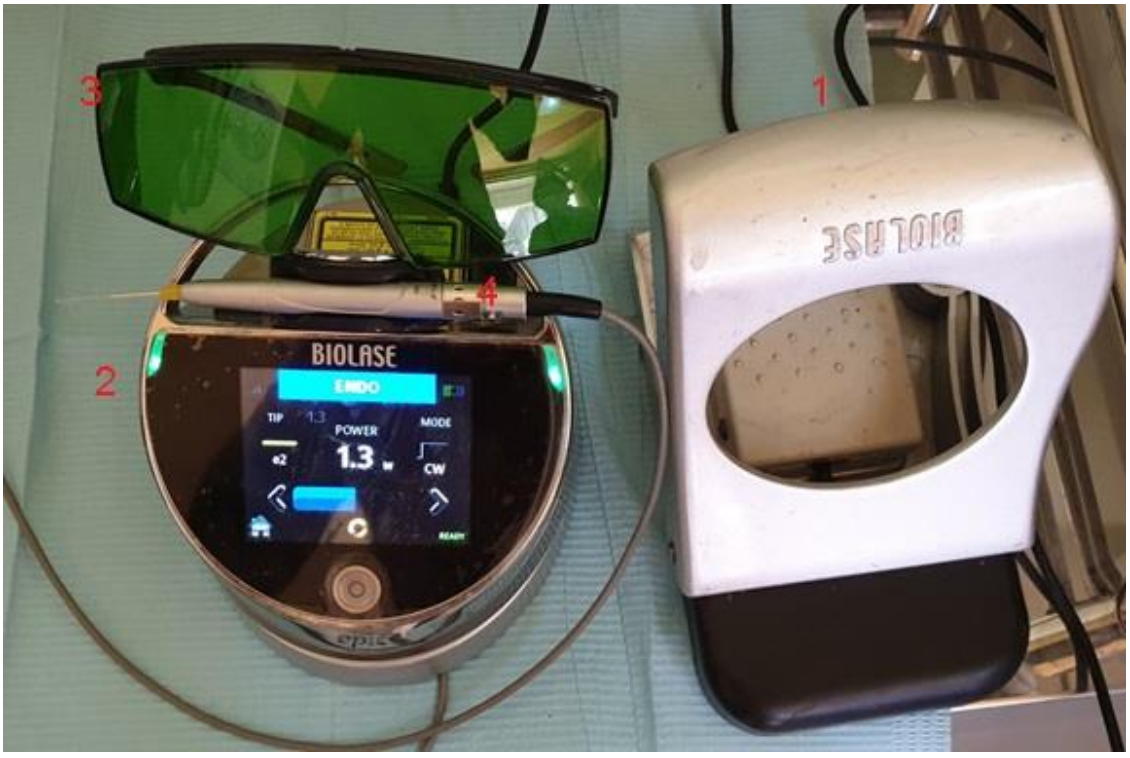


Fig. (2.9) Laser unite with its components

2- Laser classification: IV and the classification of aiming beam is cl 2.

3- Laser wavelength (λ) is 940nm ± 10 nm, and aiming beam λ is 625-670 nm.

4- Active medium: InGaAsP Semi-conductor diode.

5-power modes: continuous emission (CW) and gated pulsed modes, following table contains details of each pulsed mode.

MODE	PULSE DURATION	PULSE INTERVAL	DUTY CYCLE
CP0	10 microseconds	40 microseconds	20%
CP1	100 microseconds	200 microseconds	33%
CP2	1 millisecond	1 millisecond	50%
P3	20 millisecond	20 millisecond	50%

Table 2-1 Details of pulsed modes.

2.2.4.1 Laser parameters selection

Different laser parameters implied in regard to root canal disinfection by laser, continuous and pulsed modes with different powers, pulse durations and intervals. For diode laser the most common and effective powers used in RTC are 1 ,1.5 and 2 watts at continuous emission mode (CW) with exposure time of 5 to 10 seconds[,80,81].

A pilot study was made to test the effect laser powers on temperature elevation on the external root surface in order to select a maximum power that doesn't elicits temperature rise above the biological limit.

2.2.4.1.1 pilot study

As known, laser irradiation induces temperature elevation inside target tissue. If the temperature exceeds 7 degrees over 37°C on the external root surface damages could happen to the surrounding periodontal tissues[82,83,84]. So in this study three powers selected to test how much thay elevate temperature on the external root surface. These powers are 1, 1.3, and 1.5 watt.

The method used in this study was that a tooth specimen (prepared just like the 40 specimens used in the research) placed in stone mold and connected to a thermocouple wire at the mesial surface in the root where thinnest area in the root lies. The thermometer used is (AMPROBE TMD®-56, Everett, WA, USA, figure 2.1J) highly accurate with basic accuracy of 0.1% and its thermocouple of K-type with range of (-200°C to 1372°C) and the head diameter is 0.8 mm. the thermometer gives temperature measurement in every one second.

The thermometer was connected to computer to record temperature changes in every second figure (2.10) Shows the setup. The procedure includes inserting fiber tib (size 200 micrometer) inside root canal reacing one millimeter short from the apex then applying laser irradiation and moving the fiber in helicoid motion downward and upward for 5 seconds exposure followed by 10 seconds resting period for 4 times and in the same time the thermometer records temperature changes. The room temperature was 19.5 °C.

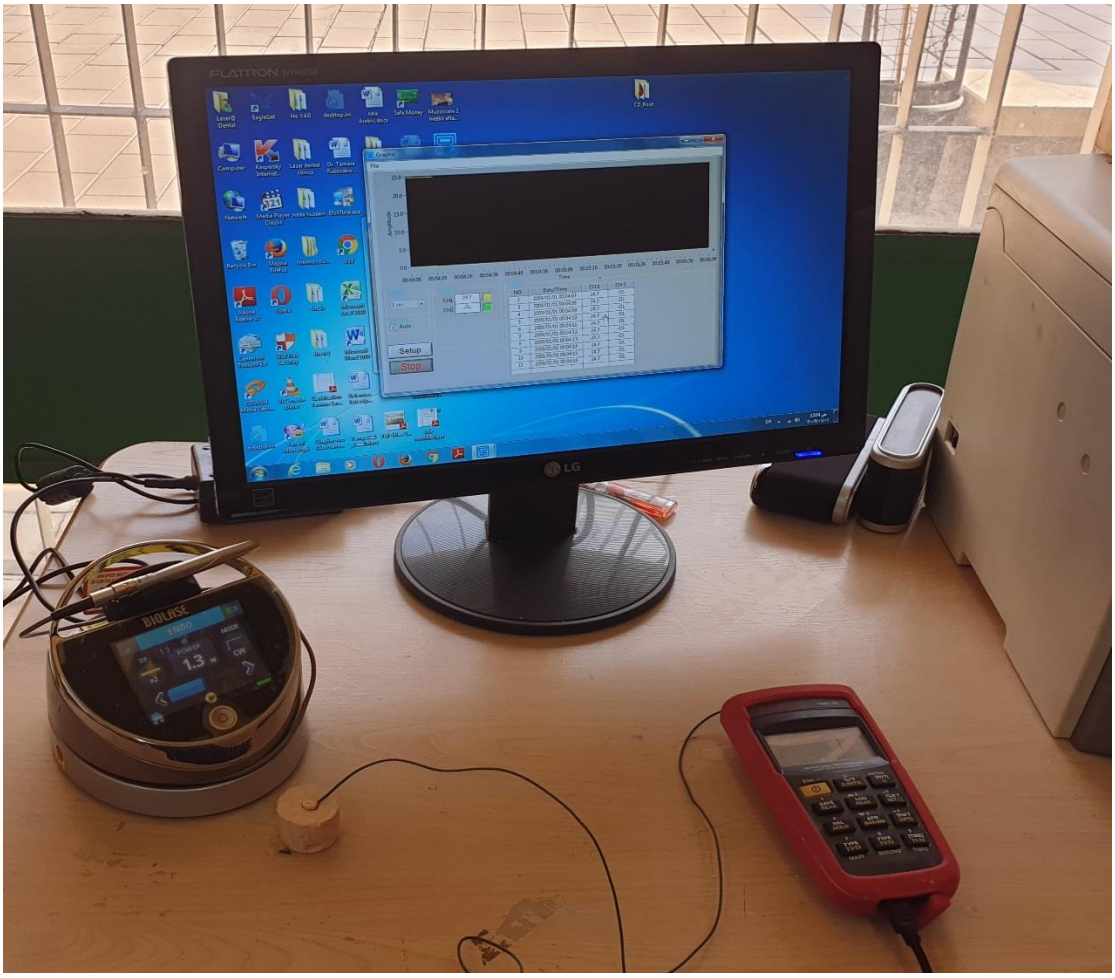


Fig. (2.10) Experimental tools for the pilot study.

The first records were for 1 watt power as in figure 2.11, before laser irradiation the temperature on the external root surface was 25.5°C and with subsequent exposures it raised up to 30.1°C so only 4.6 degrees was temperature elevation for that power level.

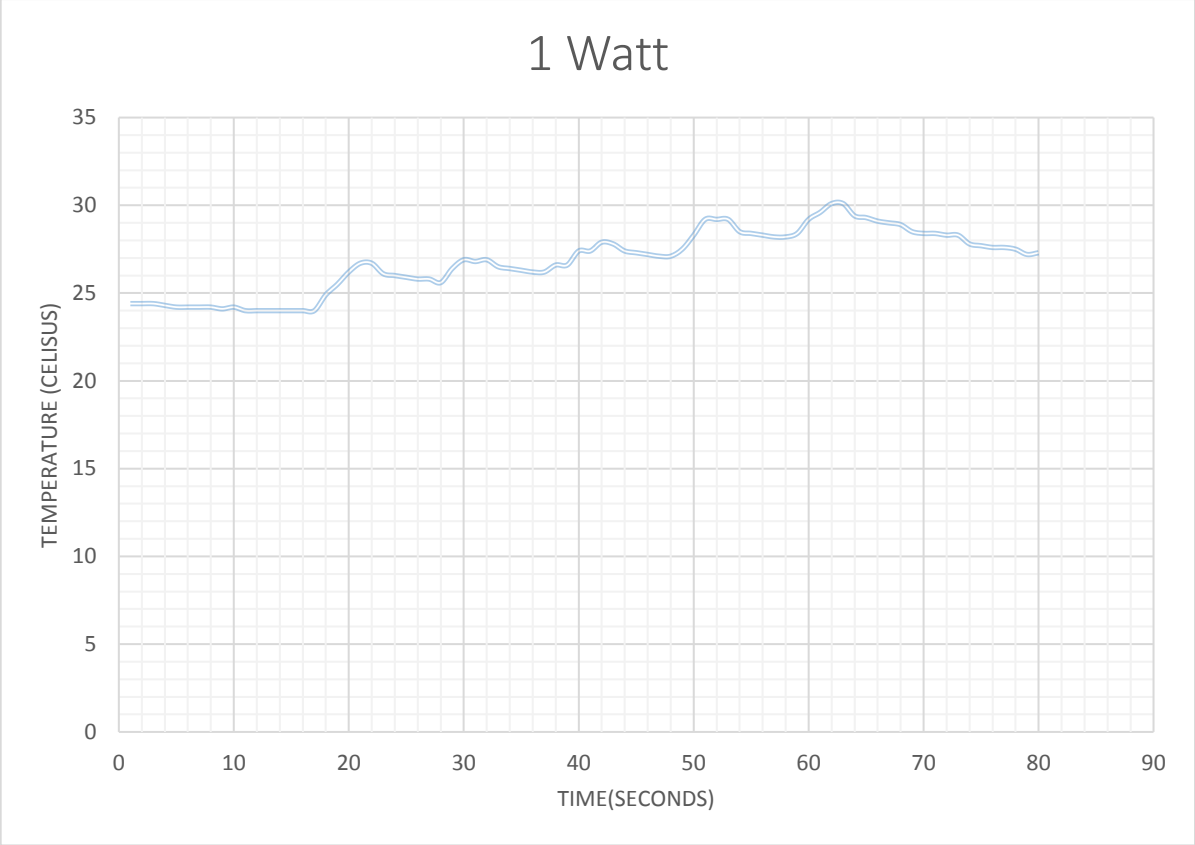


Fig 2.11 temperature changes on the external root surface during laser irradiation at 1 watt.

The second records were for 1.3 watt and in the same manner as with the previous power. The temperature elevation yielded was 6.7 degrees which is within safe limit and that was encouraging to test higher power which was 1.5 watt but unfortunately this power level elevated the temperature on the external root surface for 7.6 degrees which is harmful for the periodontium. figures 2.12 and 2.13 show temperature changes with 1.3 and 1.5 watt respectively

figure 2-12 temperature changes on the external root surface during laser irradiation at 1.3 watt.

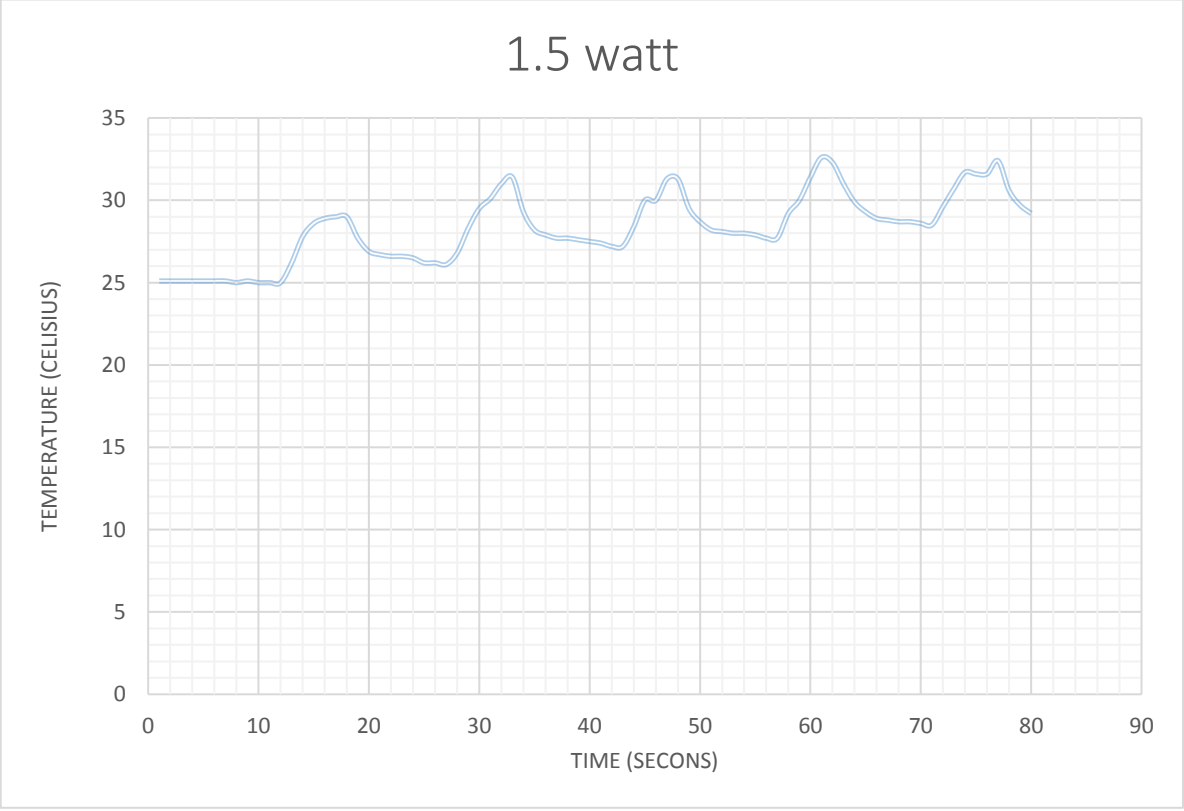


Fig. 2.13 temperature changes on the external root surface during laser irradiation at 1.5 watt.

From the results collected from all the three powers its obvious that 1.3 watt laser power would be the highest power with 5 seconds exposure time that could be safe to the tissues surrounding the tooth root as it does not cause temperature elevation of 7 degrees above the initial root surface temperature.

For this reason in the experimental disinfection 1.3 watt with exposure time 5 seconds for four times with 10 seconds interval between lasing cycles was adopted.

2.2.5 specimens grouping

After two weeks of the incubation period, all teeth specimens brought out from their test tubes and soaked in chlorohexidine bath for three minutes(figure 2.11) then plunged into sterile water for one minute and dried with sterile cotton.

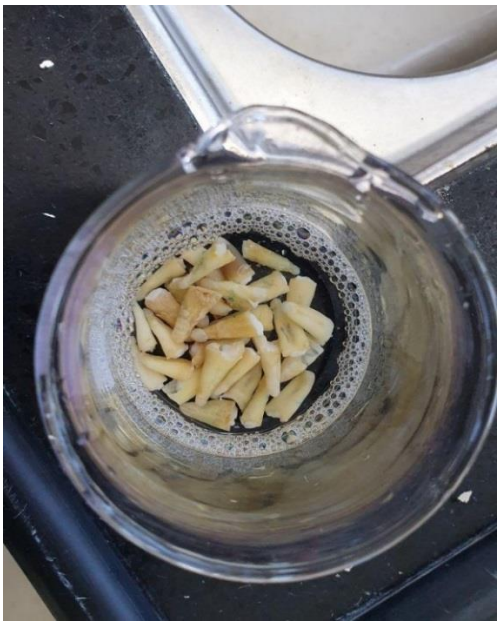


Fig. (2.11) Teeth specimens rinsed in Chx solution.

The next step was dividing the forty specimens into four groups randomly (table 2-5); control group, NaOCl group, laser group, and combination group. Each group has ten infected teeth.

Group order	Type of specimens	Number of specimens
Group A	Control	10 teeth
Group B	Laser	10 teeth
Group C	NaOCl	10 teeth
Group D	Combination (Laser+NaOCl)	10 teeth

Table (2-2) specimens grouping.

2.2.6 Experimental Root Canals Disinfection

Group B: Diode Laser

Teeth specimens in laser group were disinfected by Epic 10 diode laser as mentioned earlier with an endodontic tip (ezTip Endo, 20 mm / 200 μm) at wavelength of 940 nanometer and an output power of 1.3 watt at continuous mode (CW) as shown in figure 2.9 below.

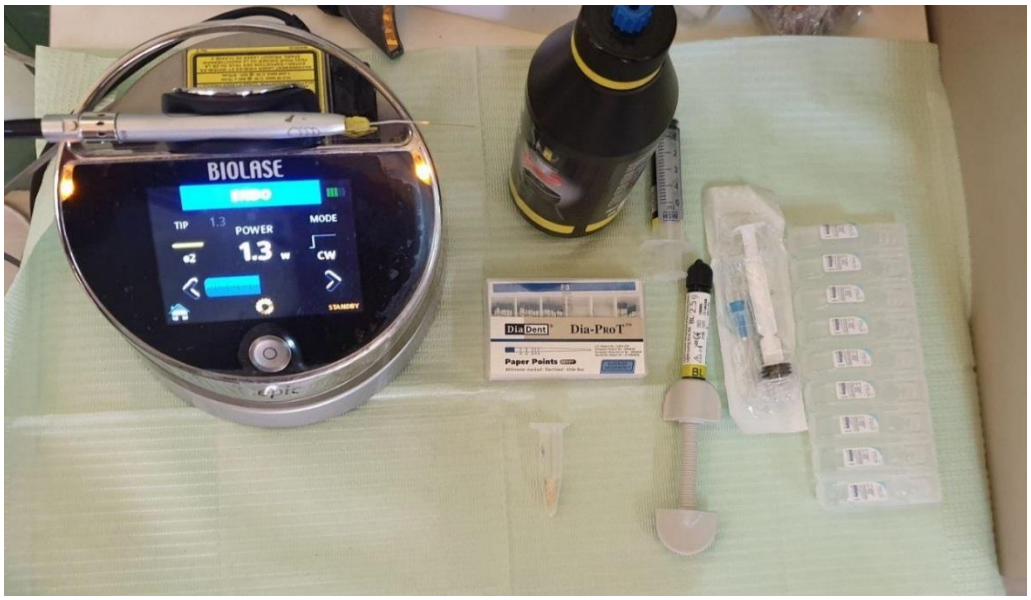


Fig. (2.14) laser setup

Each canal received 5 seconds laser exposure four times with ten seconds intervals after each exposure. The laser tip inserted directly into the canal one millimeter under the working length with helicoid motion from the apical part toward the coronal part.

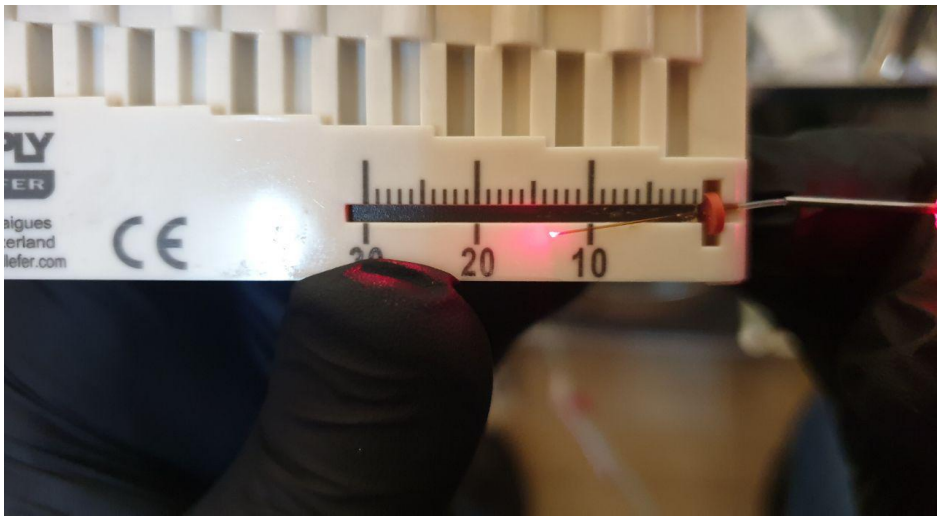


Fig. (2.13) Placing fiber's stopper at 14 mm.

After completing the procedure, each sample received 20 µl of sterile water and then sealed coronary with temporary filling.

Group C : 5.25%Sodium Hypochlorite

The specimens were irrigated with 3ml of 5.25% sodium hypochlorite for three minutes. The irrigation was performed by using a 30-gauge irrigation needle. The needles introduced passively inside root canals up to two millimeter under the working length. Figure 2.11shows the irrigation with NaOCl

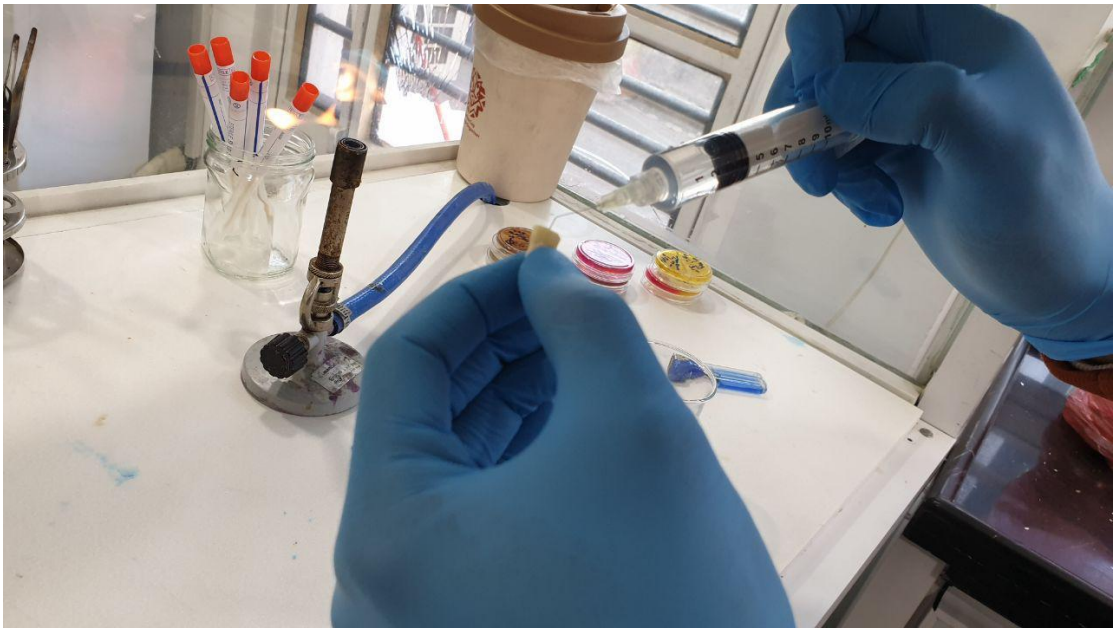


Fig. (2.14) Root canal disinfection with NaOCl

After completing the irrigation, a sterile paper point introduced into each canal to dry it during 30 seconds then a sterile water was introduced into each canal and sealed coronary with light cured temporary filling and incubated for 24 hour.

Group D : Combination

The irrigation of these specimens was similar to that in group c. after irrigation with 5.25% sodium hypochlorite laser irradiation was performed to the canals as in group b directly in the remaining sodium hypochlorite. After that a sterile water introduced in all specimens and coronary sealed and incubated for 24 hours.

2.2.7 Determination of bacterial count

After 24 hors from experimental disinfection procedures, all the 30 disinfected teeth and the 10 control teeth were opened and refilled with sterile water again to serves as transport media. Then sampling from the specimens consisted of introducing sterile #25 K-File inside the canals with circumferential filing during 30 seconds to disrupt the bacterial biofilm and collecting dentin chips.

After that sterile paper points #F3 were introduced inside the canals to collect the transport media along with dentin chips as shown in figure 2.12

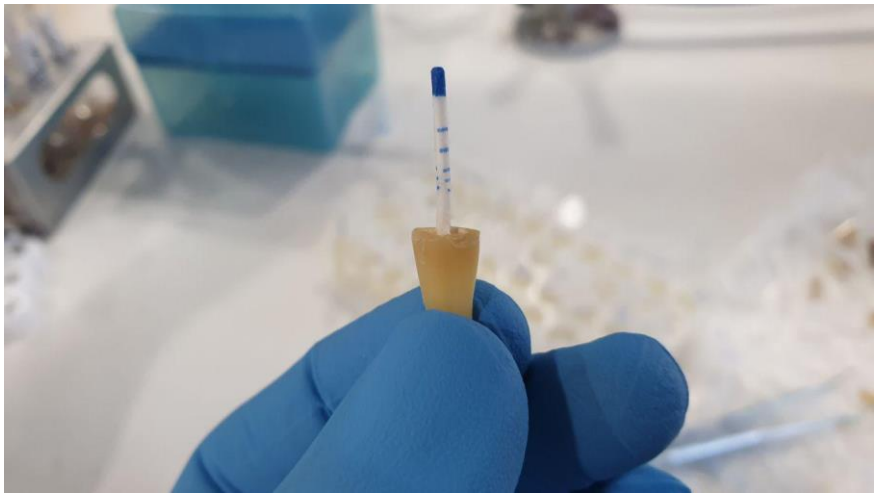


Fig.(2.15) Paper point used to collect the transport fluid

Then these paper points were placed inside Eppendorf tubes containing 2 ml of sterile lb broth and vortexed for 60 seconds (figure 2.13) then incubated at 37°C under anaerobic conditions for 24 hours.

Fig. (2.16) Vortexing paper points inside the Eppendorf tubes.

After 24 hours from all the Eppendorf tubes 0.5 ml was taken and undergo tenfold serial dilution then 0.1ml inoculated on blood agar media and after 24 hours of incubation bacterial growth was confirmed by colony forming units. Then those CFU appeared on the agar surfaces were counted manually per ml of the original specimen by using the formula:

$$\text{No. of CFU X Dilution factor} = \text{No. of CFU/ml}$$ [85,86]

Then comparisons between the control group and the other experimental groups were made to determine the reduction in CFU in each experimental group.

2.2.8 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) and maximum and minimum values.

2- Inferential Statistics:

- Shapiro-Wilks 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) is a technique used to make analysis or to study if there are statistically significant differences between means of two or more unrelated groups.
- Dunnett's T3 post hoc test. It determines the level of significance of the relationship between two sets of data.

Level of significance was determined as follow:

$P > 0.05$ not significant (NS)

$P < 0.05$ significant (S)

$P < 0.01$ highly significant (HS)

Chapter Three

Results, Discussion, and Conclusion

Chapter Three

Results, Discussion, and Conclusion

This chapter includes the results of the study and its statistical analysis, discussion, conclusion, and suggestions for future work.

3.1 Results

In this study, there were four groups:

Group A (control group) gives a predisinfection record and the results of the other groups compared with the results of this group to estimate how bacterial reduction accorded in these disinfected specimens.

- Group B, Diode 940nm laser applied alone as a bactericidal tool.
- Group C, 5.25% NaOCl utilized alone as antibacterial agent.
- Group D, specimens of this group treated with 5.25% NaOCl combined with diode laser.

Table (3-1) shows the results of group A (control group) as below:

Table (3-1) The results of group A presented as CFU/ml of E.faecalis

No.	CFU/ml
1	25000
2	28700
3	12700
4	23600
5	29500
6	22000
7	24500

8	22700
9	26100
10	28000
total	242800
mean	24280

The results of group B are shown below in **table (3-2)** where the specimens irradiated with 940nm laser.

Table (3-2) The results of group B (laser only), the results represented by CFU/ml of E.faecalis.

No.	**CFU/ml**
1	12200
2	19800
3	18400
4	16000
5	17300
6	21100
7	18100
8	19500
9	17900
10	18700
total	179000
mean	17900

Table 3-3 shows the results of group C below where all specimens treated with 5.25% NaOCl alone.

Tabel (3-3) shows results of group C, the results represent CFU/ml

No.	CFU/ml
1	7400
2	4200
3	10000
4	9500
5	0
6	7000
7	6700
8	8000
9	10700
10	4900
total	68400
mean	6840

Table 3-4 reveals the results of the last group (group D) where both sodium hypochlorite and diode laser were used.

Table 3-4 the results of group D (CFU/ml)

No.	CFU/ml
1	700
2	500
3	1100
4	900
5	800

6	1200
7	1800
8	1700
9	1300
10	1100
total	11100
mean	1110

By assuming the bacterial growth in group A (control group) measured by CFU/ml equals 100% then comparing the bacterial growth in each group with that of group A these comparisons yielding that :

in group B there was a weak antibacterial effect as the bacterial reduction was about 26.3% where diode 940nm laser used alone.

while in group C 5.25% sodium hypochlorite reduced the bacterial growth in about 71.9%, whereas the combination of NaOCl and diode laser in group D showed powerful antimicrobial activity as the bacterial reduction was 95.5%.

the next figures are blood agar plates with *E.faecalis* CFU of all the groups

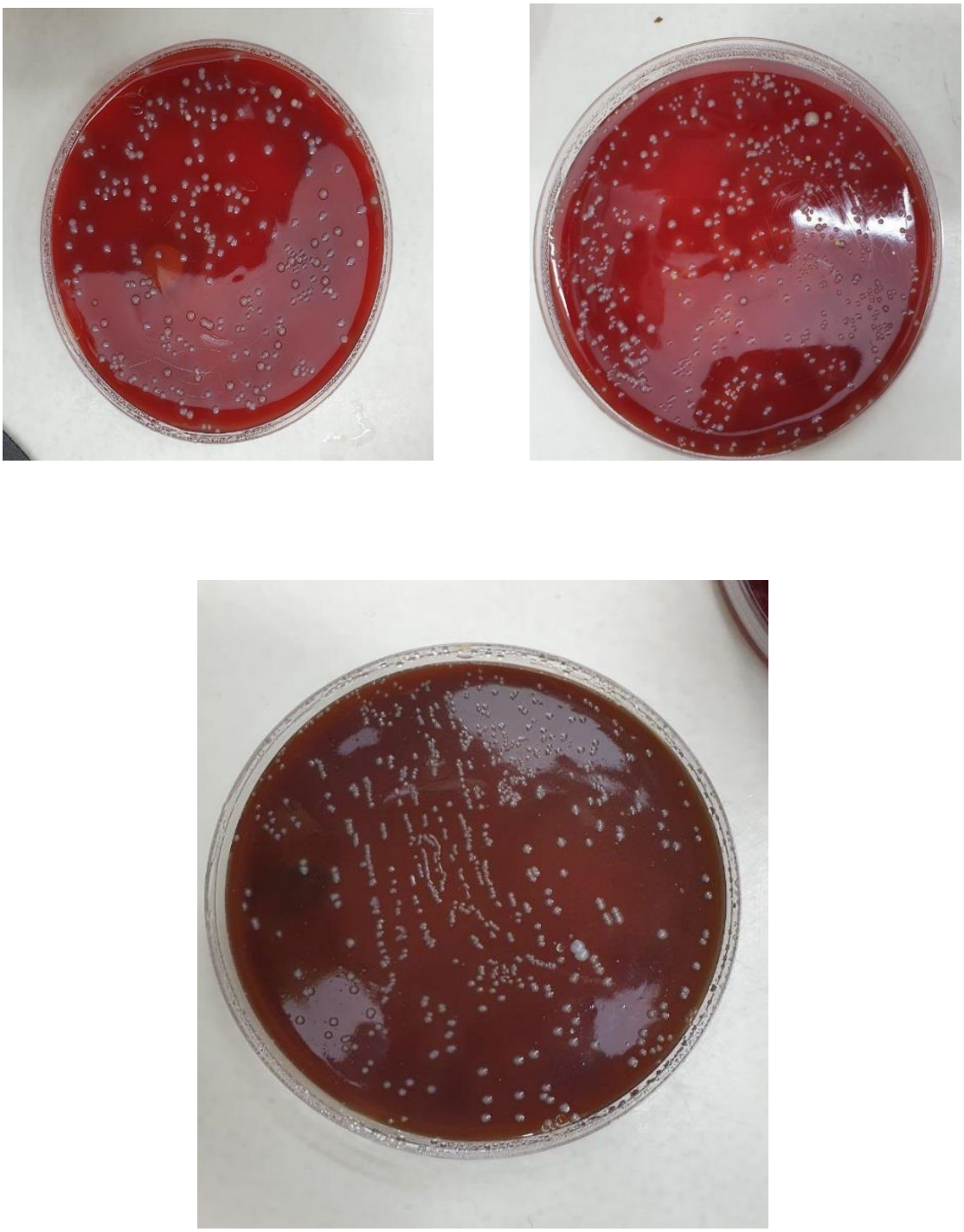


Fig. (3.1) Blood agar plates inoculated with E.faecalis from group A samples. After 24 hr. of incubation CFU appeared clearly.

Fig. (3.2) Blood agar plates inoculated with E.faecalis from group B samples. After 24 hr. of incubation CFU appeared clearly.

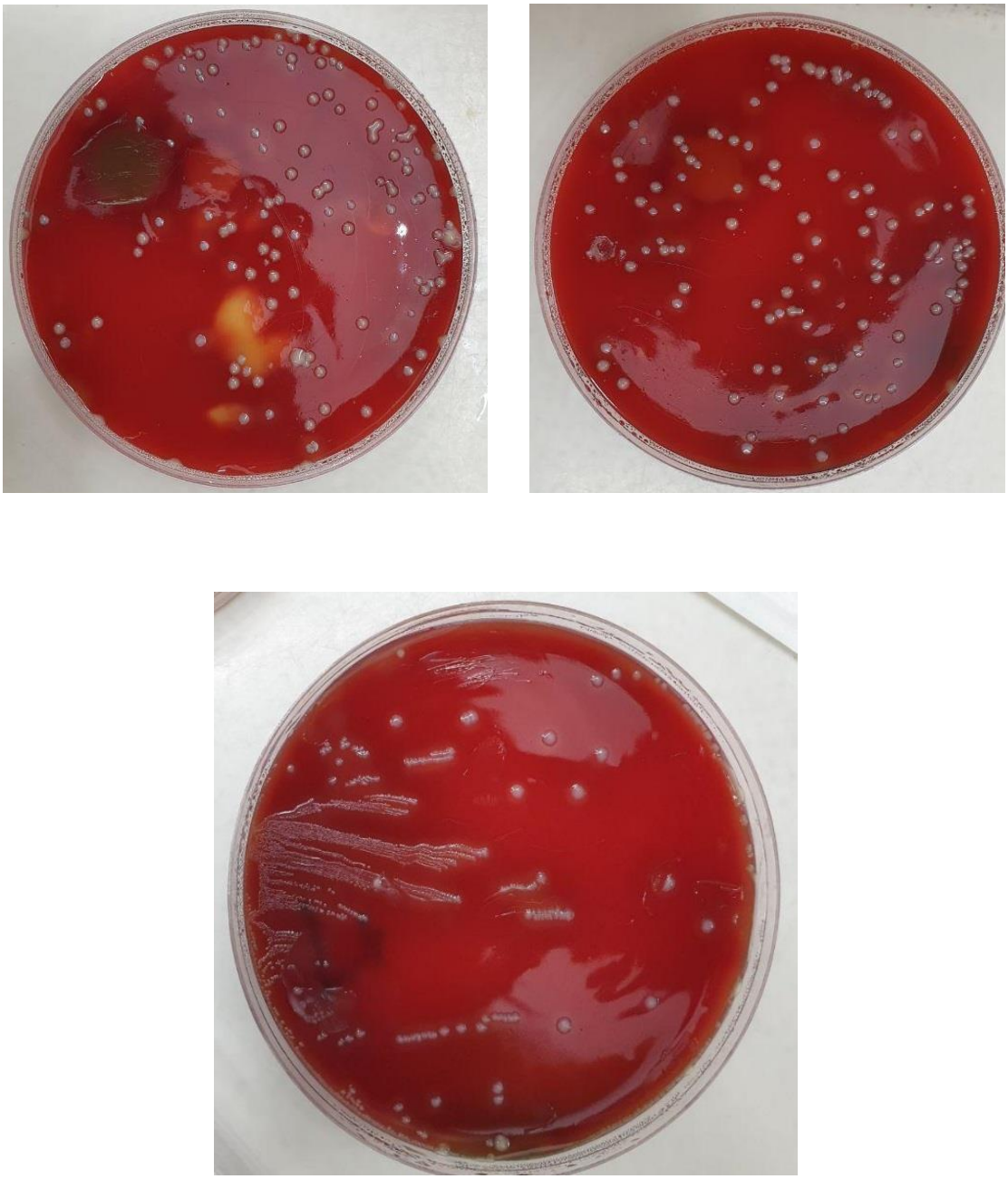


Fig. (3.3) Blood agar plates inoculated with E.faecalis from group C samples. After 24 hr. of incubation CFU appeared clearly.

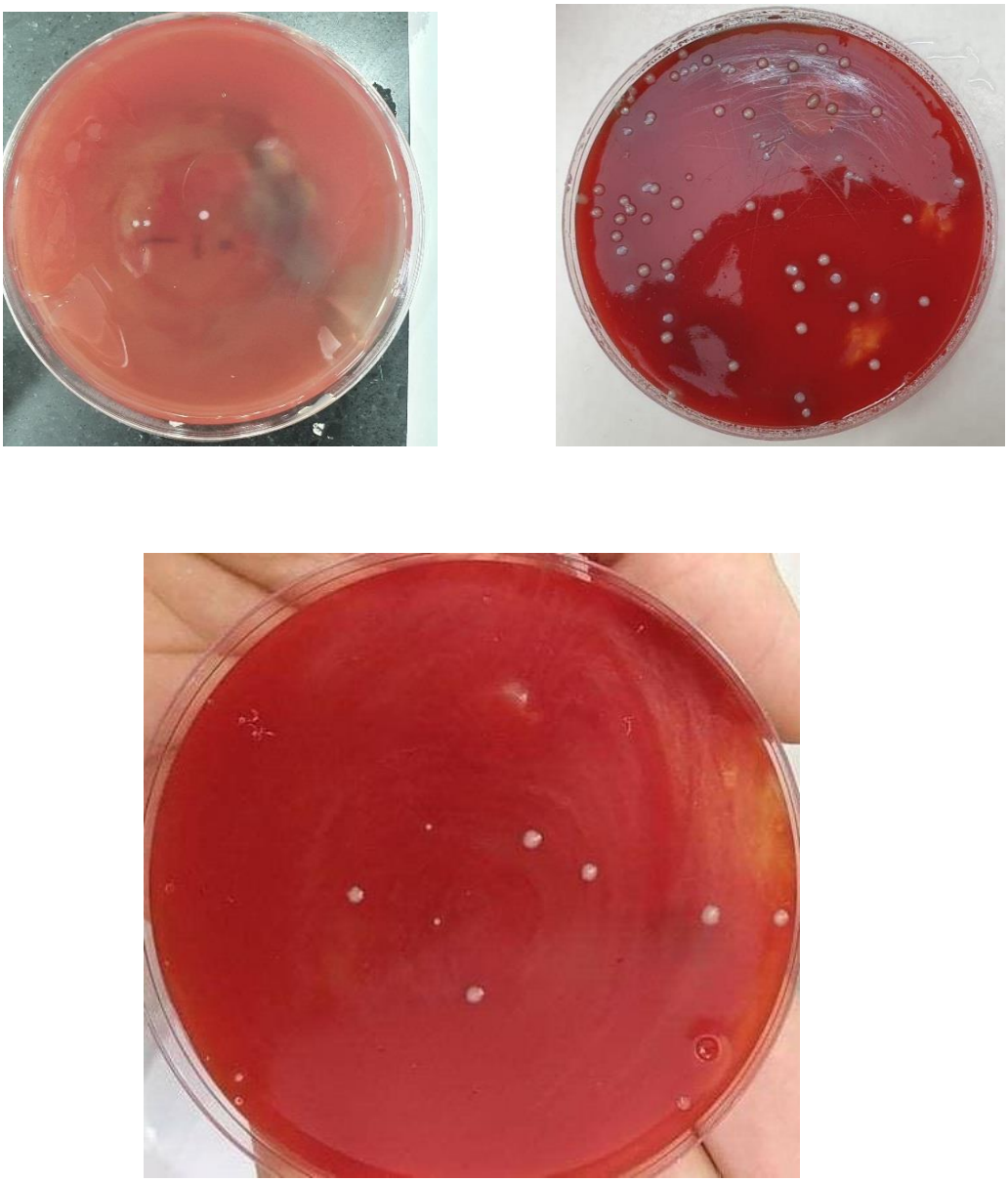


Fig. (3.4) Blood agar plates inoculated with E.faecalis from group D samples. After 24 hr. of incubation CFU appeared clearly.

3.1.1 Data distribution

First, Shapiro wilk test was made to test whether the data in all groups were normally distributed or not. For *E.faecalis* CFU in group A (which represents CFU before treatment) and in groups B,C,and D that represent CFU after different treatments table (3-5) reveals normality od distribution.

Table (3-5) Shapiro-wilk test for normality distribution of data in all groups.

Groups	Shapiro-Wilk			
	Statistic	df	P value	
Control	0.856	10	0.069	NS
Laser	0.895	10	0.191	
5.25% NAOCL	0.930	10	0.447	
Combination	0.959	10	0.774	

NS= Not significant at p>0.05

The results of Shapiro-wilk test shows that data are normally distributed in the different groups as p value in all groups is greater than 0.05 level of significance (p>0.05) and that allows to apply the conventional statistical methods either descriptive statistics represented by points and interval (such as mean, SD, SE,..) or applying applying inferential statistical methods such as parametric hypothess.

3.1.2 Descriptive statistics

Below is a table for descriptive statistics including mean, standard deviation, standard error, minimum values and maximum values for CFU/ml in all groups

Table (3-6) descriptive statistics of enterococcus faecalis CFU/ml in the control group and test groups.

CFU					
Groups	Mean	±SD	±SE	Minimum	Maximum
Control	24280.000	4793.004	1515.681	12700.000	29500.000
Laser	17900.000	2444.949	773.161	12200.000	21100.000
5.25%NAOCL	6840.000	3183.010	1006.556	.000	10700.000
Combination	1110.000	414.863	131.191	500.000	1800.000

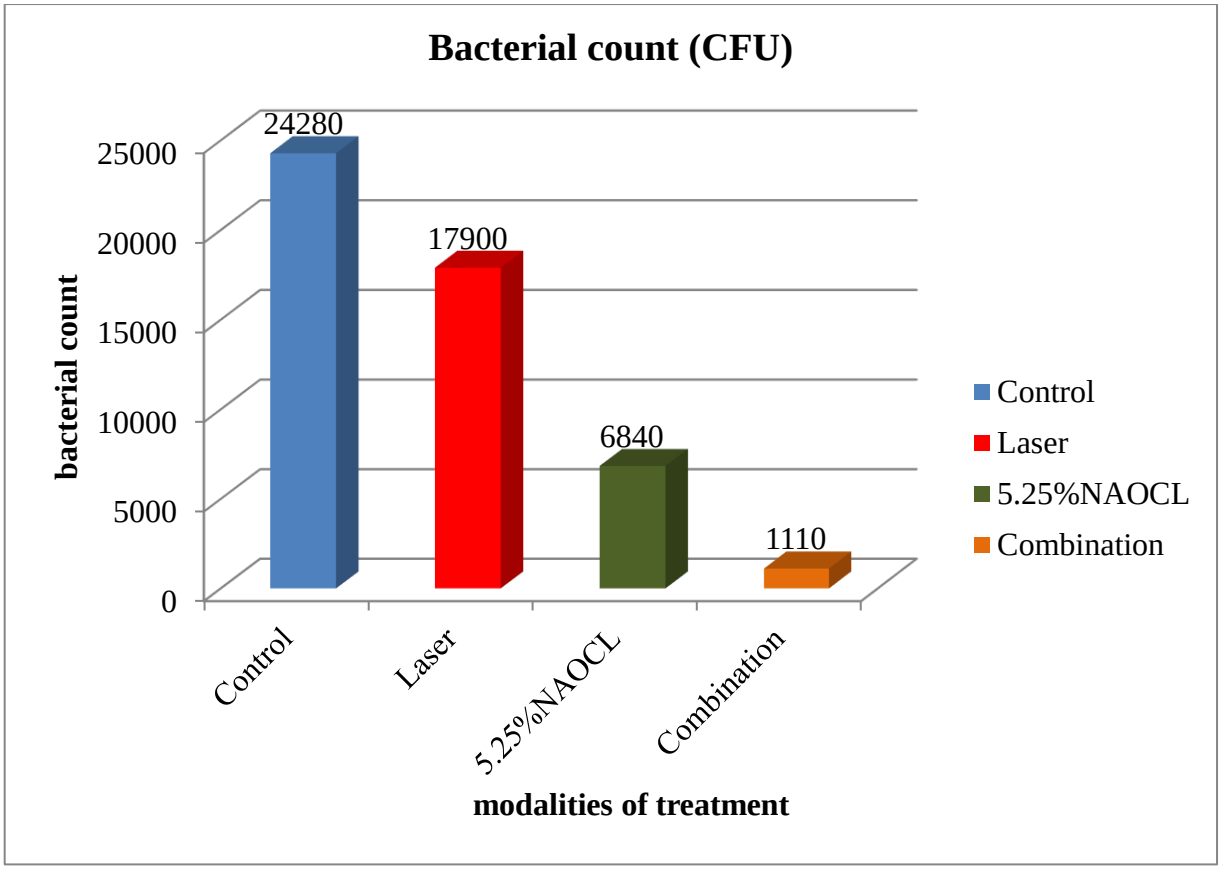


Figure (3.5) CFU means in different groups.

from the figure above it is easy to make comparisons between the means of the treated groups and the control group. The mean of combination group is the least among other groups followed by NaOCl group and laser group respectively. Its clear that the combination of NaOCl and laser radiation in the root canal has the greatest impact on *E.faecalis* biofilm.

3.1.3 Inferential statistics

A statistical test of CFU among groups using One way Analysis Of Variance (ANOVA) as shown in the table below

Table(3-7) one way ANOVA test for independent groups.

ANOVA					
CFU					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	3296918750.000	3	1098972916.667	111.985	**0.000 HS**
Within Groups	353289000.000	36	9813583.333		
Total	3650207750.000	39			

It revealed highly significant difference between groups as p <0.05

Levene staistics	f=3.220	p value=0.034 (Sig.)

Significant at p >0.034

In the next table multiple comparisons of CFU/ml between groups using (Dunnett's T3 post hoc test).

Table (3-8) testing the effectiveness of different disinfection techniques by comparing the dependent variable (CFU/ml) of all the groups.

Multiple Comparisons				
Dependent Variable: CFU/ml Dunnett's T3 post hoc test				
Groups	Groups	Mean Difference	P value	
Control	Laser	6380.000	**0.013**	**Sig.**
	5.25%NAOCL	17440.000	**0.000**	**HS**
	Combination	23170.000	**0.000**	
Laser	5.25%NAOCL	11060.000	**0.000**	
	Combination	16790.000	**0.000**	
5.25%NAOCL	Combination	5730.000	**0.002**	

This table shows that all comparisons between each pairs are highly significant using Dunnett's T3 post hoc test except between control and laser groups , the finding is significant ($p<0.05$). however, the best bactericidal effect appeared was in the combination of NaOCl and diode laser (group D) followed by sodium hypochlorite effect (group C) and the weakest antibacterial effect appeared when laser used alone inside root canal.

3.2 Discussion

The purpose of root canal treatment is to create bacteria free environment within the root canal system in order to prevent or heal apical lesions. To do so, mechanical instrumentation by files and biological disinfection by irrigation liquids with subsequent obturation should be done in every RCT.[87]

Many chemical solutions (NaOCl, CHx, EDTA,..) showed bactericidal effect against root canal microbiota however all these chemicals have its own shortages in regard to bacterial reduction and organic matter dissolution.[88]

Studies revealed that pathogenic bacteria within infected root canal can penetrate inside dentin for more than 1000 while the irrigant solution could not pass through dentin for more than 100 μm meaning that their bactericidal effect is limited for only one tenth of the bacterial depth and the remaining microorganisms are away from irrigant effect.[89]

These findings showed that the irrigation solution alone is not enough and need additional methods to boost the bactericidal effect within the canal.

In this study enterococcus faecalis biofilm was chosen to test the efficiency of 940nm diode laser and sodium hypochlorite against it due to its association with failures and the fact that this bacterial species capable of invading for more than 1100 μm inside dentinal tubules so that compromising the outcomes of root canal treatment/retreatment.[90]

The diode laser used in this study is classified as solid-state laser due to its solid active medium which composed of InGaAsP emitting radiation at wavelength 940nm+- 10nm.

The 940nm wavelength has very low absorption coefficient in water around 0.04–0.05 cm−1that gives this wavelength poor absorption by water and hydroxyapatite, however the poor absorption in dentine constituents gives the beam the characteristic of deep penetration depth (>1000 μm) allowing this wavelength to reach and destroy distant bacteria so that we used it in this study as E.faecalis known to settle deep inside the tubular system and such wavelength could be the primary antibacterial agent rather than the irrigant solution in that distant areas[90,91,92].

the photothermal effect of laser wavelength destroys bacterial cell wall. Gram negative bacteria are more radially destroyed with low powers than gram positive bacteria. This is may be attributed to the structural characteristics of bacterial cell wall.[93,94]

The 940nm laser radiation shows a weak antibacterial effect when used alone inside root canals as there was only about 26.6% reduction from the total bacterial load in group B.

This result agrees with **Ozkocak et al.** who tested the bactericidity of Nd:YAG laser and 940nm diode laser with and without photosensitizer on *E.faecalis* biofilm within human central incisors. He found that the highest surviving bacteria were collected from specimens treated by diode laser alone meaning that diode laser has a weak antibacterial effect when applied as a primary antimicrobial.[95]

Udart etal studied the antibacterial effect of 940nm diode laser on root canal bacteria and his results coincide with our results regarding the use of only laser within root canal. He found very weak bactericidal effect of the laser and concluded that the antimicrobial effect of diode laser related to its thermal effect and for such effect to be strong enough to disinfect root

canals it should elevate root canal surface temperature to around 70◦C and that is intolerable by periodontal tissues.[96]

benezra et al reported weak bactericidal effect of 810nm diode laser against planktonic culture of *E.faecalis* and he concluded that diode laser cannot considered as alternative rather than adjunctive to irrigant solution.[97]

Erben et al demonstrated that 940nm diode laser enhances the bactericidal effect of Er,Cr:YSGG on *E.faecalis* pathogens within root canal when used together and the results of this dual action is comparable to the combinatipn of NaOCl and EDTA regarding smear layer removal and bacterial disinfection. However, he reported that the depth of dentinal tubules disinfection was was superior in dual wavelength action compared with conventional irrigation.[98]

The weak antimicrobial effect achieved of the diode laser can be enhanced by different methods. For example by varying laser parameters (e.g. exposure time or pulse width) or by adding photosensitizer material such as indocyanine green and silver nanoparticles that either increase radiation absorption or triggering free radicals which in turn kill the microbes.[99,100.]

for sodium hypochlorite, different concentrations (3%, 5%, and 5.25%, 6%, …etc.) used in endodontic treatment, however invitro studies revealed that 5.25% has the best bactericidal effect against gram negative and Gram-positive oral pathogen (including yeast) with minimal toxicity.[101,102.]

The 5.25% NaOCl showed fairly good antibacterial effect when used alone against *E.faecalis* biofilm. It killed about 71.9% of the bacteria in group C.

The antimicrobial effect of sodium hypochlorite can be boosted by heating up before injecting it inside the canal to 50 c or by mechanical agitation.[103]

In the present study, the sodium hypochlorite was boosted with the diode laser and the best results achieved when 5.25% NaOCl applied together with laser radiation within the canal. This result agrees with **Castelo P *et al.*** Who worked on enterococcus faecalis biofilm cultured inside extracted human single rooted teeth and he found that the best antimicrobial effect obtained when 940nm diode laser beam combined with NaOCl applied inside the canal.[104]

According to **Olivi G** near infrared laser activate irrigation solutions by local increment of temperature. It couldn't initiate cavitations due to its poor absorption by water and hydroxyapatite.[105]

Retamozo B *et al* assumed that the time needed for NaOCl application to exert its best bactericidal effect is 40 min[106]. In this study the best bactericidal effect has been obtained when NaOCl activated by diode laser and the time required shortened to only 3 minutes as compared with the 40 minutes assumed by **Retamozo B *et al*** .

3.3 Conclusion

Based on the results of the work it can be said that 940nm diode laser is a weak antibacterial agent if used alone against *E.faecalis* as its action depends on selective absorption by pigments and in case of bacteria it is well absorbed by gram-negative bacteria with less absorption by gram-positive bacteria. However, when applied together with sodium hypochlorite it increases NaOCl activity, reducing chair time (especially irrigation time to 3 minutes) meaning that it can be best used as co-adjunctive tool in root canal disinfection.

3.4 Suggestions for future work

1- long term evaluation (more than 24 hour) of the bactericidal effect of 940nm diode laser.

2- adding photosensitizer agent to diode laser to increase the antimicrobial effect.

3- comparison between the antimicrobial effect of continuous and pulsed emission modes of 940nm diode laser at different parameters on *enterococcus faecalis* with respect to temperature elevation on the external root surface.

4- comparison between the antimicrobial effects of combination of NaOCl + diode laser and chlorohexidine + diode laser against *e.faecalis* biofilm.

5- comparative study between different wavelengths in root canal disinfection.

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خلاصة

مقدمة: السبب الرئيسي لفشل العلاج اللبي هو وجود بعض الانواع من البكتيريا داخل قنوات الجذور مثل المكورات المعوية البرازية. هذه البكتريا اكثر مقاومة لمواد التطهير مما قد تسبب التهابات مستمرة في داخل و خارج الجذر.

الهدف: الغرض من هذه الدراسة المختبرية هو امران: الاول لفحص الفاعلية المضادة للميكروبات لل ٩٤٠ نانومتر لليزر لدايود على بايوفيلم المكورات المعوية البرازية الموجودة بداخل قناة الجذور, اما الثاني فهو لتقدير ما اذا كان لليزر الدايود امكانية تعزيز فاعلية هيبوكلوريت الصوديوم بتركيز ٥,٢٥٪.

المواد و الطريقة: نماذج بكتيرية جديدة جمعت من قنوات جذور مصابة لمرضى يجرون علاج قنوات الجذور و باستخدام الفايتك تم تشخيص المكورات المعوية البرازية. بعد هذا تم تحضير اربعين سن احادي الجذر و طعموا بالزرع البكتيري ثم حضنوا لمدة اسبوعين لا هوائيا. بعد ذلك جميع عينات الاسنان قسمت الى اربع مجموعات كل مجموعة تحتوي على عشر عينات. المجموعة أ لم تتلقى اي معالجة باعتبار انها مجموعة التحكم, المجموعة ب تم تطهيرها بليزر الدايود فقط, المجموعة ج تم تطهيرها ب٥,٢٥٪هيبوكلوريت الصوديوم, المجموعة د طهرت بهيبوكلوريد الصوديوم مع اشعة اليزر. تم اخذ المسحات وذلك بادخال رأس ورقي حجم F3 في كل قناة ومن ثم وضعها في حساء لوريا-بيرتوني ثم تحضن لاربع وعشرون ساعة لياخذ بعدها قطرة من الحساء ويلطخ على وسط اغار الدم ومن ثم يتم حساب الوحدات االمنشئة للمستعمرات.

النتائج: ليزر الدايود كان العامل المضاد البكتيري الاضعف ذلك لانه قلل من الحمل البكتيري(عدد المستعمرات البكتيرية) في المجموعة ب حوالي٢٦,٣٪ بالمقارنة بالمجموعة ا بينما ٥,٢٥٪هيبوكلوريت الصوديوم اضهر تاثير مضاد بكتيري جيد على المكورات المعوية اذ انه قلل البكتريا بنسبة ٧١,٩٪ في المجموعة ج. افضل تاثير قاتل للبكتيريا حصل عندما تم تعريض هيبوكلوريت الصوديوم لاشعة االليزر اذ انهم قللو النمو البكتيري الى ٩٥,٥٪ (بالمقارنة مع المجموعة أ).

الاستنتاج: افضل استخدام لليزر الدايود هو مساعد مشارك مع سوائل تروية الاقنية الاخرى خلال اجراء تطهير الاقنية. عندما يستخدم لوحده (ليزر الدايود) فانه يعطي تأثير قاتل للبكتريا ضعيف.

وزارة التعليم العالي والبحث العلمي

جامعة بغداد

معهد الليزر للدراسات العليا

التأثير التطهيري على البكتيريا بالليزر ذو ال٩٤٠ نانومتر على المكورات المعوية البرازية في المعالجة اللبية (دراسة مختبرية)

رسالة مقدمة إلى
معهد الليزر للدراسات العليا/جامعة بغداد/ لأستكمال متطلبات
نيل شهادة ماجستير علوم الليزر/ طب الاسنان

من قبل

مصطفى محمود الانباري

بكالوريوس طب وجراحة الفم والأسنان ـ ٢٠١٥

بأشراف

استشاري جراحة الوجه والفكين و الليزر

الدكتور صلاح عبد المهدي القرطاس

١٤٤٢ هـ ٢٠٢٠ م