ผลการต้านเชื้อเอ็นเทอโรคอคคัส ฟีคาลิส ของ เออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ เปรียบเทียบกับน้ำยาล้างคลองรากฟันสองชนิด ในคลองรากฟันแท้มนุษย์ที่ถูกถอน

นายเถลิงศักดิ์ สมัครสมาน

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ANTIBACTERIAL EFFECT ON *Enterococcus faecalis* OF Er,Cr:YSGG LASER IRRADIATION COMPARED TO TWO IRRIGATING SOLUTIONS IN ROOT CANALS OF EXTRACTED HUMAN TEETH

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Endodontolc
Department of Operative Dentistry
Faculty of Dentistry
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Thesis Title	ANTIBACTERIAL EFFECT ON *Enterococcus faecalis* OF Er,Cr:YSGG LASER IRRADIATION COMPARED TO TWO IRRIGATING SOLUTIONS IN ROOT CANALS OF EXTRACTED HUMAN TEETH
By	Mr.Thalerngsak Samaksamarn
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เถลิงศักดิ์ สมัครสมาน : ผลการต้านเชื้อเอ็นเทอโรคอคคัส ฟีคาลิส ของ เออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ เปรียบเทียบกับน้ำยาล้างคลองรากฟันสองชนิดในคลองรากฟันแท้มนุษย์ที่ถูกถอน (ANTIBACTERIAL EFFECT ON *Enterococcus faecalis* OF Er,Cr:YSGG LASER IRRADIATION COMPARED TO TWO IRRIGATING SOLUTIONS IN ROOT CANALS OF EXTRACTED HUMAN TEETH) อ.ที่ปรึกษา : ผศ.ทพ.ฉันทวัฒน์ สุทธิบุณยพันธ์, อ.ที่ปรึกษาร่วม : ผศ. ทญ. ดร.อรนาฏ มาตังคสมบัติ, 93 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลการต้านเชื้อของเออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ กับน้ำยาล้างคลองรากฟันสองชนิดในคลองรากฟันแท้ของมนุษย์ที่ถูกถอน โดยศึกษาในฟันมนุษย์รากเดียว 125 ซี่ เตรียมคลองรากฟันด้วยวิธีคราวน์ดาวน์ให้มีขนาดเท่าตะไบชนิดเคเบอร์ 50 แบ่งฟันทั้งหมดโดยการสุ่มเป็น 4 กลุ่ม ๆ ละ 30 ซี่ ที่เหลืออีก 5 ซี่ใช้เป็นกลุ่มควบคุมภาวะปลอดเชื้อ ภายหลังการทำให้ปราศจากเชื้อ คลองรากของฟันทุกซี่ยกเว้นในกลุ่มควบคุมภาวะปลอดเชื้อถูกเพาะเชื้อ เอ็นเทอโรคอคคัส ฟีคาลิส ความเข้มข้น 1 x 10^8 CFU/มิลลิลิตร ปริมาณ 10 ไมโครลิตร ในอุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 48 ชั่วโมง กลุ่มแรกใช้เป็นกลุ่มควบคุมผลลบซึ่งไม่ได้รับการล้างคลองราก กลุ่มที่สองและสามได้รับการล้างคลองรากฟันเป็นเวลา 10 นาที ด้วยโซเดียมไฮโปคลอไรต์ 2.5% และ คลอเฮกซิดีน 2 % ปริมาณ 5 มิลลิลิตรตามลำดับ และกลุ่มสุดท้ายได้รับการฉายด้วย เออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ กำลัง 1.5 วัตต์จำนวน 4 รอบ ๆ ละ 10 วินาที ทดสอบเชื้อที่เหลืออยู่ในทุกกลุ่ม โดยใส่น้ำเกลือลงไปในคลองรากฟันและใช้ตะไบชนิดเคเบอร์ 50 ขูดโดยรอบผนังคลองรากฟัน หลังจากนั้นดูดน้ำเกลือในคลองรากฟัน นำไปเพาะบนวุ้นเลี้ยงเชื้อทันทีที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 24 ชั่วโมง นับจำนวนโคโลนีและนำข้อมูลที่ได้มาวิเคราะห์ด้วยสถิติ One-Way ANOVA ที่ระดับนัยสำคัญ 0.05 ผลการศึกษาพบว่าค่าเฉลี่ยของปริมาณแบคทีเรียที่เหลือในกลุ่มควบคุมผลลบที่ไม่ได้รับการล้างคลองรากมีจำนวนมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เมื่อเปรียบในกลุ่มที่ฉายด้วยเลเซอร์และกลุ่มที่ได้รับการล้างด้วยน้ำยาล้างคลองรากฟันทั้งสองชนิดพบว่าค่าเฉลี่ยของปริมาณแบคทีเรียที่เหลืออยู่ในกลุ่มที่ฉายด้วย เออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ มีจำนวนสูงกว่ากลุ่มที่ได้รับการล้างด้วยน้ำยาล้างคลองรากฟันทั้งสองชนิดอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) แต่ในกลุ่มที่ได้รับการล้างด้วยโซเดียมไฮโปคลอไรต์ 2.5% และ คลอเฮกซิดีน 2 % ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) จากการทดลองนี้สรุปได้ว่า เออร์เบียม โครเมียม อิตเทรียม สแกนเดียม แกลเลียม การ์เนต เลเซอร์ สามารถลดปริมาณแบคทีเรียในคลองรากฟันลงได้ในระดับหนึ่ง แต่มีประสิทธิภาพด้อยกว่าน้ำยาล้างคลองรากฟัน โซเดียมไฮโปคลอไรต์ 2.5% และ คลอเฮกซิดีน 2 %

ภาควิชาทันตกรรมหัตถการ
สาขาวิชาวิทยาเอ็นโดดอนต์
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ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4976108732 : MAJOR ENDODONTOLOGY

KEY WORDS : CHLORHEXIDINE, *Enterococcus faecalis*, Er,Cr:YSGG, LASER IRRADIATION, SODIUM HYPOCHLORITE

THALERNGSAK SAMAKSAMARN: ANTIBACTERIAL EFFECT ON *Enterococcus faecalis* OF Er,Cr:YSGG LASER IRRADIATION COMPARED TO TWO IRRIGATING SOLUTIONS IN ROOT CANALS OF EXTRACTED HUMAN TEETH. THESIS ADVISOR : ASST. PROF. CHANTAVAT SUTTHIBOONYAPAN, THESIS CO-ADVISOR : ASST. PROF. ORANART MATANGKASOMBUT, Ph.D., 93 pp.

The objective of this study was to compare the antibacterial effect of Er,Cr:YSGG laser irradiation with two irrigating solutions in root canals of extracted human teeth. One hundred and twenty-five extracted single rooted teeth with straight roots were chosen. The canals were enlarged with K files to size 50 using crown-down technique and randomly assigned into four experimental groups of 30 teeth each and 5 teeth for sterility control group. After sterilization, all roots except the sterility control group were inoculated with 10 µl of $1x10^8$ CFU/ml of *Enterococcus faecalis* for 48 h at 37 °C. The first group was used as a negative control group receiving no treatment. The second group and third group were irrigated with 5 ml of 2.5% NaOCl solution and 2% CHX solution for 10 min, respectively. The last group was irradiated with the Er,Cr:YSGG laser at 1.5 W output power with no air and water using four lasing cycles of 10 s each. After treatment, the canals were filled with sterile normal saline solution and were circumferentially filed with H-file size 50. The content in the canals was then transferred using a micropipette, plated on TSA agar immediately and incubated at 37 °C for 24 h. The colony forming units were counted, and the quantitative results were subjected to an One- Way ANOVA. The mean number of viable colonies in the negative control group was significantly higher than the other groups ($p < 0.05$). Comparing among the treated groups, the mean Log colony forming units (Log CFU) value obtained after Er,Cr:YSGG laser irradiation was statistically significantly higher than those of 2.5 % NaOCl and 2% CHX groups ($p < 0.05$). However, there was no significant difference between the 2.5 % NaOCl and 2% CHX groups ($p > 0.05$). It can be concluded that Er,Cr:YSGG laser irradiation can reduce the viable microbial population in root canals to a certain extent but is less effective than irrigating with 2.5 % NaOCl and 2% CHX solutions.

Department Operative Dentistry
Field of study Endodontology
Academic year 2007

Student's signature.....
Advisor's signature.....
Co-advisor's signature.....

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LIST OF ABBREVIATIONS

NaOCl	=	Sodium Hypochlorite
CHX	=	Chlorhexidine
E. faecalis	=	*Enterococcus faecalis*
TSB	=	Tryptiene Soy Broth
TSA	=	Tryptiene Soy Agar
Er,Cr:YSGG	=	Erbium Chromium Yttrium Scandium Gallium Garnet
CFU	=	Colony-forming unit
Log CFU	=	Logarithmic scale Colony-forming unit

CHAPTER I

INTRODUCTION

1.1 Background of Present Study

Bacterial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions (Kakehashi *et al*., 1965). Therefore, the main goal of endodontic treatment is the elimination of bacteria found inside the dental root canal. The persistence of bacteria in the root canal system after endodontic treatment may cause persistant inflammation in the periradicular tissue and often leads to failure (Molander *et al*., 1998). Accepted treatment procedures to eliminate the infection include a combination of chemical cleaning involving irrigation with a disinfectant and mechanical instrumentation. The most popular irrigating solution is sodium hypochlorite (NaOCl). It is an effective antimicrobial agent (Bystrom *et al*., 1983) and an excellent organic solvent for vital, necrotic and fixed tissues. Sodium hypochlorite dissolves proteins, forming chloramines residues on the remaining peptide fragments, thus not only aiding in debridement but also contributing to antimicrobial action of the free chlorine. Furthermore, it inactivates the sulfhydryl groups of bacterial enzymes by forming hypochlorous acid (Caliskan *et al*., 1994). However, it is highly irritating to periapical tissues, especially at high concentrations (Becking 1991; Ercan *et al*., 2004). Chlorhexidine gluconate has been recommended as a root canal irrigant and medicament. It is a potent antimicrobial agent has a low grade of toxicity. Chlorhexidine seems to act by adsorbing onto the cell wall of

microorganisms and causing the leakage of intracellular component (Gomes *et al*., 2003). Antimicrobial substantivity of chlorhexidine in the root canal system has been reported to last from 48 hours to 21 days (White *et al*., 1997). However, chlorhexidine is unable to dissolve pulp tissue and may remain on canal walls, obstructing the dentinal tubules (Kuruvilla *et al*.,1998).

Infected root canals have a complex microbial flora that may also penetrate to the dentine to variable depths up to 300 μm (Ando *et al.,* 1990) or more (Horiba *et al*. 1990). In addition, bacteria like *Enterococcus faecalis* are known to be one of the predominant bacteria in teeth which root canal therapy failed and appear highly resistant to medicament used during treatment (Bystrom *et al*., 1985; Sjogren *et al.,* 1997) .

Despite the use of antimicrobial chemicals for irrigation, the existence of accessory canals, anastomoses and fins creates a three-dimensional network that makes the complete elimination of debris and achievement of a sterile root canal system difficult with conventional irrigation solutions (Bystrom *et al*., 1981). The apical third of the instrumented root canal remains partially untreated with insufficient removal of debris and necrotic soft tissue (Haikel *et al*., 1988).

New approaches to eliminate the infection from root canal systems include the non-instrumented technique and use of laser technology (Kimura *et al*., 2000; Lussi *et al*., 1995). The antibacterial effect of a laser beam is based on thermal properties of the laser tissue interaction (Rooney *et al*., 1994). Laser radiation has potential to aid in endodontic treatment (Stabholz *et al*., 2004). It was suggested that besides the improved removal of debris and smear layer, dental lasers could provide greater accessibility to formerly unreachable parts of the tubular network because of their enhanced

penetration into dentinal tissues (Klinke *et al*., 1997; Vaarkamp *et al*., 1995) and consequently may have antimicrobial effects to aid in the reduction of bacteria in the root canal (Hardee *et al*., 1994; Mehl *et al*., 1999; Moshonov *et al*., 1995).

The disinfecting ability of difference types of lasers in laboratory-based models has been studied from several aspects using the Er:YAG (Dostalova *et al.,* 2002; Jelinkova *et al*., 1999; Mehl *et al.,* 1999; Moritz *et al*., 1999; Rooney *et al*., 1994; Schoop *et al*., 2004; Schoop *et al*., 2002), the Nd: YAG (D'Ercole *et al.,* 2004; Fegan *et al*., 1995; Folwaczny *et al*., 2002; Gutknecht *et al*., 1996; Hardee *et al*., 1994; Moritz *et al*., 2000; Moshonov *et al*., 1995; Piccolomini *et al*., 2002; Ramskold *et al*., 1997; Rooney *et al*., 1994; Schoop *et al*., 2004) the Ho:YAG(Gutknecht *et al*., 1997; Moritz *et al*., 1999), the CO_2 (Dederich *et al*., 1990; Le Goff *et al*., 1999; Zakariasen *et al*., 1986), the Excimer (Folwaczny *et al*., 1998; Stabholz *et al*., 1993), the Nd:YAP (Blum *et al*., 1997), the Diode (Gutknecht *et al*., 2004; Gutknecht *et al*., 2000) and the Alexandrite systems (Jelinkova *et al*., 1999). All lasers were found to have variable bactericidal effects.

As a result of advances in the field of laser-assisted endodontics a new hard tissue laser, the erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser at 2.78 μm has become available. The Er,Cr:YSGG laser system uses hydrokinetic energy-the laser energy heats the air and water directly in front of the atomized water molecules with the aim of accelerating them to a higher speed. As a result of this hydrokinetic energy, the Er, Cr:YSGG laser may have a greater ability to disinfect root canals.

Previous studies on the Er,Cr:YSGG lasers seemed to focus on caries removal and cavity prevention, and little is known about its bactericidal

effectiveness (Hadley *et al*., 2000). Moreover, there are only a few studies that compared the antibacterial efficacy of the Er,Cr:YSGG laser irradiation in infected root canal with standard irrigating solutions (Eldeniz *et al*., 2007: Wang *et al*., 2007) .

1.2 Research questions

Is the antibacterial effect of Er,Cr:YSGG laser irradiation different from 2.5 % NaOCl and 2% CHX when used in the root canals infected with *Enterococcus faecalis*?

1.3 Research objectives

To compare the antibacterial effect of Er,Cr:YSGG laser irradiation with 2.5 % NaOCl and 2% CHX when used in the root canals infected with *Enterococcus faecalis*.

1.4 Hypothesis

Null Hypothesis H_0: The number of remaining bacteria after using Er,Cr:YSGG laser irradiation, 2.5 % NaOCl or 2 % CHX as an irrigant in the root canals infected with *Enterococcus faecalis* is not different.

Alternative hypothesis H_A : The number of remaining bacteria after using Er,Cr:YSGG laser irradiation, 2.5 % NaOCl or 2 % CHX as an irrigant in the root canals infected with *Enterococcus faecalis* is different at least one pair.

1.5 Keywords

Antibacterial, Chlorhexidine, *Enterococcus faecalis,* Laser irradiation, Sodium hypochlorite

1.6 Research design

Laboratory experimental research

1.7 Limitations of research

The experimental design is an *in vitro* study using extracted human single rooted teeth. Effects of the interventions in this experiment cannot be completely generalized to the population. Therefore, further experiments have to be investigated.

1.8 Benefits

1. To evaluate the antimicrobial efficacy of using Er,Cr:YSGG laser irradiation as a new method for disinfection in the root canal system.
2. To obtain basic knowledge for further studies in clinical situation.

1.9 Ethical consideration

There was no ethical problem because human teeth used in this study were extracted for variety clinical reasons with patient's informed consent at the Department of Oral Surgery, Faculty of Dentistry, Chulalongkorn University.

CHAPTER II

LITERATURE REVIEW

Microbial etiology of apical periodontitis

Apical periodontitis is an inflammatory process in the periradicular tissues caused by microorganisms in the necrotic root canal (Bergenholtz 1974; Kakehashi *et al*., 1965). Kakehashi *et al*.(1965) took normal rats and germ-free rats and exposed their pulps. All normal rats had non-vital, necrotic pulps and periapical abscesses by day 8. The germ-free rats never lost pulp vitality. No granulomas or abscesses formed. Dentinal bridges began to form by day 14, with complete healing of the exposures by day 28, even with gross food impaction in the endodontic access hole. Spangberg(1988) stated that the importance of infection now is accepted as the major factor for the development of periradicular inflammatory disease. Sundqvist *et al*.(1998) found that most cases of endodontic failure are thought to involve a continuing infection of the root canal system. Nair *et al.* (1990) and Sjogren *et al*.(1997) concluded that the most probable reason for failure of endodontic treatment is the presence of a persisting infection. Schilder (1974) stated that the success of endodontic treatment depends on the dentist's ability to clean and disinfect the complex canal system three dimensionally, then to fill and seal this space completely.

Enterococcus faecalis

Enterococcus faecalis is a facultative Gram-positive bacterium. It has been considered one of the most resistant species in the oral cavity and one possible cause of post-treatment disease after root canal treatment (Sundqvist

et al., 1988; Molander *et a*l., 1998). It could infect dentinal tubule up to 800 μm from the root canal (Haapasalo and Orstavik 1987). Several studies have reported their low susceptibility to irrigant solution (Gomes *et al*., 2001; Vianna *et al*., 2004) and intra canal medicaments (Bystrom *et al*., 1985). Moreover, sodium hypochlorite and chlorhexidine have prove to be effective against *Enterococcus faecalis in Vitro*, but they require direct contact (Sassone 2003). It has been reported that enterococci are frequently isolated from obturated root canals of teeth that exhibit chronic periapical pathology (Sundqvist *et al*., 1998). These facts indicated that *Enterococcus faecalis* has a pathogenic role in chronic endodontic treatment failure.

The goal of endodontic treatment

There is a widely accepted view that cleaning and shaping of the root canal system is the most important step toward sterility of the canal. The necessary elements in the control of endodontic infection are (Haapasalo *et al*., 2003):

- host defense system, systemic antibiotic therapy (only occasionally and with special indications)
- instrumentation and irrigation
- locally used intracanal medicaments between appointments
- root canal filling and coronal restoration

The goal of instrumentation and irrigation is to remove and/or kill all microorganisms in the root canal system, and to neutralize any antigenic/biological potential of the microbial components that remain in the canal.

Antibacterial irrigating solution

The use of irrigating solutions is an important part of effective chemomechanical preparation. Root canal cleansing is supported by copious irrigation.

Properties of ideal irrigating solution (Stabholz *et al.*, 1993)

1. Tissue or Debris solvent. In regions inaccessible to instruments, the irrigant could dissolve or disrupt soft tissue or hard tissue remnants to permit their removal.
2. No toxicity. The irrigants should be non injurious to periradicular tissues.
3. Low surface tension. This property promotes flow into tubules and into inaccessible areas. Alcohol added to an irrigant decreases surface tension and increases penetrability, whether this enhances is unknown.
4. Lubricant. Lubrication helps instruments to slide down the canal. All liquids have this effect, some more than others.
5. Sterilization (or at least disinfection).
6. Removal of smear layer.
7. Availability.
8. User friendliness.
9. Moderate cost.
10. Convenience.
11. Adequate shelf life.
12. Ease of storage.
13. No stain.

Function of an irrigant

1. Gross debridement.
2. Elimination of microbes.
3. Dissolution of remnant pulp tissue.
4. Lubricant.

Sodium hypochlorite (NaOCl)

NaOCl is the most popular and the most widely advocated irrigant. It can fulfill the first four actions of the ideal irrigant. It enhances bacterial elimination and facilitates removal of necrotic tissue and dentine chips from the root canal. In water, NaOCl ionizes to produce Na+ and the hypochlorite ion (OCl^-) which establishes an equilibrium with hypochlorous acid (HOCl). Hypochloric acid has been found to disrupt oxidative phosphorylation and other membrane-associated activities (Barrette *et al*., 1989). It has also been indicated that DNA synthesis is sensitive to HOCl (McKenna *et al*., 1988). NaOCl is used in concentrations varying from 0.5% to 5.25%. A 2.5% solution is commonly recommended.

NaOCl is best known for its strong antibacterial activity. It kills bacteria very rapidly even at low concentrations. Waltimo *et al.* (1999) showed that the resistant microorganism, *Candida albicans* was killed *in vitro* in 30 seconds by both 5% and 0.5% NaOCl, whereas concentrations 0.05% and 0.005% were too weak to kill the yeast even after 24 hours of incubation. The high susceptibility of *C. albicans* to NaOCl was recently also verified by Radcliffe *et al.* (2004). However, Vianna *et al.* (2004) reported that 0.5% NaOCl required 30 minutes to kill C. *albicans,* whereas 5.25% solution killed all yeast cells in 15 seconds. Gomes *et al.* (2001) tested *in vitro* the effect of various concentrations against *E. faecalis.* The microbe was killed in less than 30

seconds by the 5.25% solution, while it took 10 and 30 min for complete killing of the bacteria by 2.5% and 0.5% solutions, respectively.

Bystrom and Sundqvist (1985) showed that although 0.5% NaOCl, with or without ethylenediamine-tetra-acetic acid (EDTA) improved the antibacterial efficiency of preparation compared with saline irrigation, all canals could not be rendered bacteria free even after several appointments. The same authors could not show any significant difference in antibacterial efficiency *in vivo* between 0.5% and 5% NaOCl solutions. Siqueira *et al*. (2002) also demonstrated the superior antibacterial affect against root canal bacteria of hypochlorite in comparison with physiological saline. However, they showed no difference among 1%, 2.5%, and 5%NaOCl solutions similar to Bystrom & Sundqvist (1985).

NaOCl has been criticized for its unpleasant taste, relative toxicity, and its inability to remove smear layer (McComb *et al*., 1976; Spangberg *et al*., 1988). Pashley *et al.* (1985) compared the biological effects of mild and strong NaOCl solutions and demonstrated greater cytotoxicity and caustic effects on healthy tissue with 5.25 % NaOCl than with 0.5 % and 1 % solutions. Chang *et al.* (2001) also showed the relationship between the concentration and cytotoxicity of NaOCl. Therefore, it might be recommended to use 0.5-1 % NaOCl for canal irrigation instead of the 5.25 % solution.

<u>Chlorhexidine</u> (CHX)

CHX gluconate is a broad-spectrum antibacterial agent. It has been in use for a long time in dentistry because of its antimicrobial properties, its substantivity, and its relatively low toxicity. Despite the advantages of CHX, its activity is pH dependent and is greatly reduced in the presence of organic matter (Russell *et al*., 1993). It has a wide antimicrobial spectrum and is

effective against both Gram-positive and Gram-negative bacteria as well as yeasts, while mycobacterial and bacteria spores are resistant to CHX (Russell 1996).

CHX is probably the most widely used biocide in antiseptic general products. It is able to permeate the cell wall or outer membrane and attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrance. High concentrations of CHX cause coagulation of intracellular constituents (McDonnell *et al*., 1999).

However,an *in vitro* study by Gomes *et al*. (2001) demonstrated marked difference in the killing of enterococci by CHX and NaOCl. Only the highest concentration of 5.25 % NaOCl killed *E.faecalis* rapidly in 30 seconds, while with a lower concentration (0.5-4%), 5-30 minutes were required for complete killing to occur. CHX digluconate, on the other hand, killed *E.faecalis* cells in 30 seconds or less in concentrations of 0.2-2 %. The result was later supported by Oncag *et al.* (2003) and Vianna *et al*.(2004), who also showed *in vitro* CHX to be superior to NaOCl in killing of *E.faecalis* and *Staphylococcus aureus*. The same study revealed that CHX in a gel form required a much longer time to kill *E. faecalis* than the corresponding concentration in a liquid.

CHX lacks the tissue-dissolving ability which is one of the obvious benefits of NaOCl. While *in vitro* studies have demonstrated the antibacterial effect of CHX against *E. faecalis* to be superior to that of NaOCl. There is no *in vivo* study yet available that would confirm the better activity of CHX against this resistant species in the infected root canal. Nevertheless, there is no doubt that CHX gluconate in concentrations between 0.2% and 2% offers a good alternative for root canal irrigation with potent antimicrobial activity. Future studies of CHX combinations are needed to establish whether these

could give additional advantage in the fight against resistant root canal microbes.

Laser in Dentistry

History

The ruby laser was first developed by Maiman in 1960. A laser is a device which transforms light of various frequencies into a chromatic radiation in the visible, infrared, and ultraviolet regions with all the waves in phase capable of mobilizing immense heat and power when focused at close range. Stern & Sognnaes(1965) and Goldman *et al.*(1964) were the first to investigate the potential uses of the ruby laser in dentistry. They began their laser studies on hard dental tissues by investigating the possible use of a ruby laser to reduce subsurface demineralization. Indeed, they did find a reduction in permeability, to acid demineralization, of enamel after laser irradiation.

The first laser use in endodontics was reported by Weichman & Johnson (1971) who attempted to seal the apical foramen *in vitro* by means of a high power-infrared (CO_2) laser. Although their goal was not achieved, sufficient relevant and interesting data were obtained to encourage further study. Subsequently, attempts were made to seal the apical foramen using the Nd:YAG laser (Weichman *et al.*, 1972).

Fundamentals of lasers

The word laser is an acronym for Light Amplification by Stimulated Emission of Radiation. A brief description of each of those five words begins to explain the unique qualities of a laser instrument, and in turn, becomes the foundation for further elaboration of the uses of lasers in dentistry.

Light is a form of electromagnetic energy that behaves as a particle and a wave. The basic unit of this energy is called a photon, or a *particle* of light. Laser light possesses three additional characteristics:

- Collimination
- Coherency
- Efficiency

Amplification is a part of process that occurs inside the laser. Identifying the components of a laser instrument is useful in understanding how light is produced.

Stimulated emission has its basis in the quantum theory of physics.

Radiation refer to the light wave produced by the laser as a specific form of electromagnetic energy.

Lasers are generically named for the material of the active medium which can be a container of gas, a crystal, or a solid-state semiconductor.

- Gas : Argon, CO2
- Solid-state semiconductor : Al, Indium, Er, Nd

Terminology

All of the laser used in dentistry feature parameter that are adjustable by the clinician. Each wavelength has photon energy. The laser light photon produce a tissues effect, know in basic physics as work. Energy is ability to perform work and is expressed as joules or millijoules. Power is the measurement of the work completed over time and is measure in watts(Coluzzi 2004).

1 watt = 1 joule/second

watts x seconds = joules

Laser energy and tissue temperature

The thermal effect of laser energy on soft tissue primarily revolves around the water content of the tissue and the temperature rise of the tissue. When the tissue temperature reaches approximately 60°C, proteins begin to denature without any vaporization of the underlying tissue (Coluzzi 2004).

Table 1 Effect of temperature on target tissue (Coluzzi 2004).

Tissue Temperature (°C)	Observed Effect
37-50	Hyperthermia
>60	Coagulation, protein denaturation
70-90	Welding of tissue
100-150	Vaporization
>200	Carbonization

Laser wavelengths used for dentistry

The following are brief descriptions of the available laser devices that have dental applications. The laser is named according to its active medium, wavelength, delivery system, emission modes, tissue absorption, and clinical applications.

Table 2 Laser used in dentistry

Laser Type	Wavelength
ArF Excimer	193 nm
KrF Excimer	248 nm
XeCl Excimer	308 nm
Frequency – Doubled Alexandrite	377 nm
Krypton Ion	407 nm
Argon Ion	488, 514.5 nm
Dye	507 – 510 nm
Frequency – Doubled Nd :YAG (KTP)	532 nm
Diode (Low Level)	600 – 908 nm
Gold Vapor	628 nm
Argon – Pumped Dye	630 nm
Copper Vapor Pumped Dye	630 nm
Helium – Neon	632 nm
Ruby	694.3 nm
Diode (GaAlAs, GaAs)	800-830, 904-950 nm
Nd:YLF	1.053 μm
Nd:YAG	1.064 μm
Nd:YAP	1.34 μm
Ho:YAG	2.12 μm
Er:YSGG	2.79 μm
Er:YAG	2.94 μm
Free Electron	3.0, 6.1, 6.45 μm
Carbon Dioxide (CO_2)	9.3,9.6,10.6 μm

ArF = Argon Fluoride; Er:YAG = Erbium:Yttrium Aluminum Garnet; Er:YSGG = Erbium:Yttrium Scandium Gallium Garnet; GaAlAs = Gallium Arsenide; Ho:YAG = Holmium:Yttrium Aluminum Garnet; KrF = Krypton Flouoride; KTP = Potassium Titanyl Phoshate; Nd:YAG = Neodymium:Yttrium Aluminum Garnet; Nd:YAP = Neodymium:Yttrium Alunimun Perovskite; Nd:YLF = Neodymium:Yttrium Lanthanum Fluoride; XeCl = Xenon Chloride (Sulewski 2000)

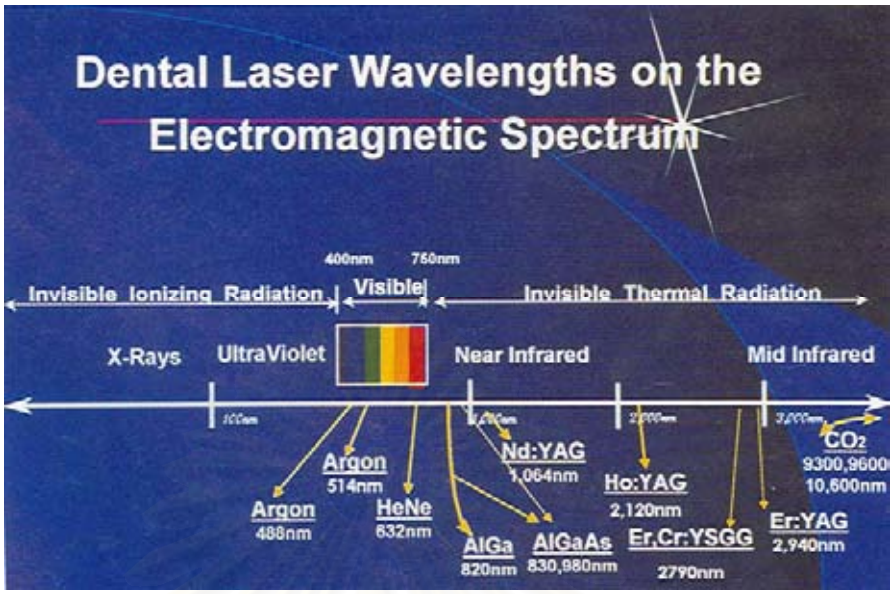

Figure1 Currently available dental laser wavelengths on the electromagnetic spectrum. Note all of the wavelengths are nonionizing (Coluzzi 2004)

Argon

Argon laser is a laser with active medium of argon gas. The energy is delivered through fiberoptic in continuous-wave and gated-pulse modes and are visible in human eye. This laser has two emission wavelengths. The 488 nm is blue in color, and 514 nm is blue-green color (Coluzzi 2004).

The 488 nm emission is the wavelength which needed to activate camphoroquinone and commonly used photoinitiator that causes polymerization of the resin in light-cured composite restorative materials. There are studies demonstrated increasing in strength of laser cure resin when compared with visible light-cured resins (Powell *et al.,* 1995). Using the argon light for this purpose results in a much shorter curing time compared with

conventional dental lights with the advantage of having an excessive amount of photons to ensure proper cure of the material. Moreover, the argon laser also can be used with other dental materials such as light-activated impression paste and light-activated bleaching gels.

The 514 nm wavelength has its peak absorption in red pigment such as hemoglobin, hemosiderin and melanin. It can use in surgical field with excellent hemostatic capabilities. Using in contact with the tissue, the argon laser would be ideally suited to treat of acute inflammatory periodontal disease and highly vascularized lesions such as a hemangioma (Finkbeiner 1995).

Both wavelengths are not well absorbed in dental hard tissues and are poorly absorbed in water. The poor absorption into enamel and dentin is advantageous when using this laser for cutting and sculpting gingival tissues because there is no interaction and no damage to the tooth surface during those procedures. They also can be used as an aid in caries detection. When the argon laser light illuminates the tooth, the diseased or carious area appears a dark orange-red color and is easily discernible from the surrounding healthy structures (Kutsch 1993).

Carbon dioxide (CO_2)

The CO_2 laser is a gas-active medium laser that delivered through a hollow tube-like wave-guide in continuous or gated-pulse mode. The wavelength of 10,600 nm or 10.6 μm which is in the end of the mid-infrared invisible nonionizing portion of the spectrum (Coluzzi 2004). It is well absorbed by water and rapidly soft tissue removed and has a shallow depth of penetration into tissue which is important when treating mucosal lesions for example. It is also useful in cutting dense fibrous tissue due to the highest absorption in hydroxyapatite of any dental laser about 1000 times greater than the erbium series of lasers. Because this wavelength was one of the earliest

used in general medical surgery, there are numerous published papers verifying its efficacy (Pogrel 1989; Israel 1994; Convissar and Gharemani 1995).

The CO_2 laser is delivered in a hollow waveguide with a handpiece. The laser energy is conducted through the wave-guide and is focused onto the surgical site in a noncontact fashion. The loss of tactile sensation is a disadvantage for the surgeon but the tissue ablation can be precise with careful technique. Large lesions can be treated easily using a simple back-and-forth motion. The procedure proceeds quickly because there is no need to touch the tissue. The current delivery system technology somewhat limits its hard tissue applications but ongoing research shows favorable results for surface modification and strengthening of tooth enamel for increased caries resistance (Featherstone *et al.*1996).

Diode

Diode laser is a solid active medium. It is a solid-state semiconductor laser that uses some combination of aluminum, gallium, and arsenide to change electric energy into light energy. The available wavelengths for dental use range about 800 to 980 nm which is in the beginning of the near-infrared invisible nonionizing part of the spectrum. The laser delivers energy through fiber optically in continuous-wave and gated-pulse modes and used ordinarily in contact with the tissue (Coluzzi 2004). The optic fiber needs to be cleaved and prepared before initial use and occasionally during long procedures to ensure the efficient operation of the laser. Glasslike tips can be placed on the end of the fiber for certain applications. The wavelength range puts this laser into the invisible nonionizing infrared radiation portion of the electromagnetic spectrum. All of the wavelengths of this laser are similar to argon and very well absorbed by pigmented tissue but is not quite rapid hemostasis as argon

laser. These lasers are relatively poorly absorbed by tooth structure so that soft tissue surgery can be performed safely in close proximity to enamel, dentin and cementum. The diode is an excellent soft tissue surgical laser indicated for cutting and coagulating gingiva and mucosa and for soft tissue curettage or sulcular debridement (Coluzzi 2002; Moritz *et al.,* 1997). Using the continuous emission mode must be taken care when because of the rapid thermal increase in the target tissue. The major advantage of the diode lasers is use of a smaller size instrument. The units are portable and compact, are easily moved with minimum setup time and are the lowest-priced lasers currently available.

Nd:YAG

Nd:YAG is a solid active medium, a crystal of yttrium-aluminum-garnet doped with neodymium and deliver through fiberoptic in a free-running pulsed mode. It is used most often in contact with the tissue (Coluzzi 2004). It was the first laser designed exclusively for dentistry and it is the laser with the largest market share. The emission wavelength is 1064 nm which in the near-infrared invisible nonionizing part of the spectrum. It is highly absorbed by pigmented tissue and is about 10,000 times more absorbed by water than an argon laser. Using the high peak powers of a free-running pulse emission with relatively long tissue cooling time, common clinical applications are for cutting and coagulation of dental soft tissues with good hemostatic ability. The free-running pulse mode allows the clinician to treat thin or fragile tissue with a reduction in heat buildup in the surrounding area. Nd:YAG laser energy is absorbed slightly by dental hard tissue but there is little interaction with sound tooth structure and allowing tissue surgery adjacent to the tooth to be safe and precise. There are numerous published clinical case studies showing effective periodontal disease control using this laser for sulcular debridement (White

1991; Neill and Mellonig 1997; Raffetto 2001). There is also a useful clinical application in vaporizing pigmented surface carious lesions without removing the healthy surrounding enamel (White *et al*. 1993).

The fiber usually is used bare-ended in contact with the tissue. During use, the fiber end needs to be cleaved and cleaned; otherwise the laser light rapidly loses its effectiveness. When used in a noncontact defocused mode, this wavelength can penetrate several millimeters into soft tissue which can be used advantageously for delivering the laser energy to the inner surface for an ulcerated lesion.

Ho:YAG

Ho:YAG is a solid active medium, a crystal of yttrium-aluminum-garnet doped with holmium, and deliver through fiberoptic in contact with the tissue in free-running pulsed mode, The emission wavelength is 2120 nm and also in the near-infrared invisible nonionizing part of the spectrum (Coluzzi 2004). Its absorption by water is 100 times greater than Nd:YAG, and it has many soft tissue surgical uses. This laser can remove soft tissue rapidly and the optic fiber affords good access, precision, and tactile feedback because that tissue contains a large amount of water. Because this laser has good absorption by water and is produced in a pulsed mode, the tissue ablation at the surgical site can proceed at an efficient rate, and collateral thermal damage can be avoided, The pulse rate, or the amount of pulses of laser energy per second is rather low compared with a Nd:YAG laser and the resulting incisions can be somewhat jagged edged. Clinically, this rough surface may manifest itself only on tissue that is more fibrous but the healing result would still be acceptable. The optical fiber which is similar to the diode and Nd:YAG lasers needs to be cleaned and cleaved periodically during surgery. A Ho laser has little react

with pigmented tissue (Kautzky *et al.*, 1997). Its hemostatic ability is decreased because of its lower absorbency into hemoglobin and other similar pigments. The laser's absorbency by tooth structure is low which allows tissue surgery in close proximity to enamel, dentin, or cementum to proceed safely. The Ho laser frequently is used for arthroscopic surgery on the temporomandibular joint and had many medical application (Hendler *et al.,* 1992).

Er,Cr:YSGG and Er:YAG

Er,Cr:YSGG laser is an active medium of a solid crystal of yttrium-scandium - gallium-garnet that is doped with erbium and chromium. While Er:YAG is an active medium of a solid crystal of yttrium-aluminum-garnet that is doped with erbium The emission wavelengths of Er,Cr:YSGG is 2790 nm and Er:YAG is 2940 nm. Both of these wavelengths are near the boundary of the near-infrared and mid-infrared, invisible, and nonionizing portion of the spectrum and similar in properties. Both of these lasers are delivered fiberoptically in the free-running pulsed mode. The fibers are air-cooled and have a larger diameter than the other lasers mentioned, making the delivery system somewhat less flexible. At the end of the fiber, a handpiece and small-diameter glass tips concentrate the laser energy down to a convenient surgical size, approximately 0.5 μm. The technical challenge in building an optic fiber system stems from the fact that the wavelength's size cannot be transmitted easily along the glass molecules so that the fiberoptic bundle is costly and can be fragile (Coluzzi 2004).

These two wavelengths have the highest absorption in water of any dental wavelength and have a high affinity for hydroxyapatite. A portion of the laser energy couples into the hydroxy radical in the apatite crystal (Eversol and Rizoiu 1995). The water that is bound to the crystalline structures of the

tooth absorbs the laser light readily and easily. The vaporization of the water within the mineral substrate causes a massive volume expansion and this expansion causes the surrounding material literally to explode away (Rechmann *et al.,* 1998). The free-running pulse mode provides the peak power to facilitate the explosive expansion, and laboratory studies indicate that the pulpal temperature of the treated tooth may actually decrease by 5^{o} C during laser treatment (Fife 1998).

These lasers are ideal for caries removal and tooth preparation when used with a water spray. The sound tooth structure can be preserved better when the carious material is being ablated. The increasing of water content of dental caries allows the laser to interact preferentially with that diseased tissue (Keller *et al*., 1997). The healthy enamel surface can be modified for increased adhesion of restorative material by exposing it to the laser energy (Martinez-Insua *et al.,* 2000). The current indication for use of these lasers dictates that they not be used for removal of amalgam or other metal. The advantage of these lasers for restorative dentistry is that a carious lesion in close proximity to the gingiva can be treated, and the soft tissue recontoured with the same instrumentation. For endodontics, removal of pulp tissue and dentine is easily accomplished with these wavelengths (Stabholz *et al.,* 2003).

Laser in Endodontics

The main areas of use for lasers in dentistry are surgery, periodontics, and operative dentistry. However, there has also been considerable interest in the potential of lasers in endodontics. Most laser delivery systems currently on the market have the ability to deliver laser energy to the root canal system. Conventional fiberoptic cables (Nd:YAG, diode) can be placed directly into root canals. Waveguide and air-cooled fiberoptic delivery systems (Er:YAG,

Er:Cr:YSGG, CO_2) have handpiece attachments that can deliver laser energy into the root canals. The goals of endodontic therapy may be summarized as follows (Coluzzi *et al*., 2004):

1. Debridement of the canal
2. Instrumentation of the canal
3. Removal of the smear layer
4. Sterilization of the canal
5. Sealing of the main and all accessory canals

Sterilization of the canal

The purpose of using a laser for endodontic treatment to sterilize a root canal system, any wavelength would work. The criterion for selection of an ideal wavelength for endodontic therapy is the ability to deliver bactericidal energy to the root canal system. Every laser currently on the market has been proved to be bactericidal. More specifically, lasers have been shown to be bactericidal in root canals *in vivo* and *in vitro* (Gutknecht *et al*., 1997).

Fegan & Steiman (1995) reported that Nd: YAG laser was effective in inhibiting the growth of *Bacillus stearothermophilus* in artificially infected root canals *in vitro.* Moshonov *et al*. (1995) assessed the efficacy of Nd :YAG laser irradiation in disinfecting the root canal system infected for 60 min with an overnight culture of *E.faecalis.* While Nd : YAG laser irradiation significantly reduced the number of bacteria, it was inferior to NaOCl irrigation, which effectively disinfected the canals. Similar results were obtained by Blum *et al.* (1997) with Nd:YAP laser on root canals infected with *Streptococcus mitis.* Excellent antibacterial efficiency against *Enterococcus faecalis* was reported by Gutknecht *et al*.(1997), who determined the bactericidal effect of a holmium : yttrium-aluminumcomparison garnet (Ho: YAG) laser on root canals

infected with this species *in vitro:* on average, 99.98% of the bacteria injected in the root canal could be eliminated. Le Goff *et al.* (1999) evaluated the effectiveness of a CO_2 laser in root canal disinfection and reported an average 85% decrease in the colony-forming units in the laser rotary treated group. However, irrigation with 3%NaOCl was superior to the CO_2 laser treatment. Contrary to this result, Kreisler *et al.* (2003) had indicated that complete sterility of the root canal can be obtained with a CO_2 laser microprobe coupled onto a special handpiece attached to the delivery fiber.

Schoop *et al.* (2002) studied the effect of an Er: YAG laser in 220 extracted human teeth and reported a good antibacterial effect, and that the bactericidal effect was dependent on the applied output power and specific for the different species of bacteria investigated. However, sterility could not be obtained predictably. Piccolomini *et al.* (2002) evaluated the efficacy of the pumped diodium-Nd: YAG laser in sterilizing contaminated root canals after hand instrumentation, 30 teeth were inoculated with *Actinomyces naeslundii* and 30 teeth with *Pseudomonas aeruginosa* and incubated for 24 h. The results indicated an average of a 34.0% decrease in colony forming units for *A. naeslundii* and 15.7% for *P. aeruginosa* with the 5 Hz/15 s laser treatment, and a decrease of 77.4% for *A. naeslundii* and 85.8% for *P aeruginosa* with the 10 Hz laser frequency. However, both results were inferior to NaOCl, as no bacteria were detected in the canals treated with 5.25% NaOCl, used as a control. Mehl *et al.* (1999) also investigated the antimicrobial properties of Er: YAG-laser radiation in root canals. The canals of 90 freshly extracted anterior teeth were enlarged mechanically sterilized and randomly divided into subgroups. The root canals were superficially contaminated by inoculating them with *Escherichia coli* or S. *aureus* for 2 hours. Bacterial counts were reduced to 0.034-0.130% from the original inoculum with the time

and energy parameters used. A corresponding reduction (0.020-0.033%) was obtained with 1.25% NaOCl solution.

Er: Cr,YSGG Laser in endodontics

It is believed that the mechanism that make the YSGG laser effective in cutting hard tissue is based on the theory of hydrokinetics which is the rapid expansion and vaporization of water molecules that have been irradiated with YSGG laser energy. These excited molecules and the ancillary YSGG laser light that is transmitted to the target tissue combine with the excited water molecules to ablate crystalline oral hard tissues that contain high amounts of hydroxyl apatite and water, as well as oral soft tissue. With some minor adjustments to the power output of the laser system along with the air and water spray, the YSGG laser has also proven effective in the surgical excision and removal of oral osseous tissue. The YSGG wavelength has proven effective for the cutting of hard tissue, soft tissue and osseous tissue.

In 2002, the YSGG wavelength was the first laser approved for complete root canal therapy. In 2003, the YSGG laser also cleared by the FDA for endodontic surgery. With these clearances the YSGG began to experience growing acceptance and implementation by endodontists worldwide. Applications include treatment of dental hypersensitivity, posttreatment temporomandibular joint (TMJ) pain, pulp abscess, pulpotomy, pulpectomy, cleaning and shaping and most importantly better disinfection of the root canal system so as to come closer to a true sterilization prior to obturation. The YSGG laser emits a low level of energy into the canal system and penetrates into the dentin tubules and the root canal system that are not accessible by hand, rotary instrumentation or chemical rinses. Studies have demonstrated that the YSGG wavelength effectively eliminates the smear layer (Ali *et al*., 2005; Schoop *et al*., 2007). It also penetrates almost 1 mm into

dentin, thereby eradicating microorganisms deep into the dentin tubules and the root canal system. By using standard hand and rotary instrumentation to widen the canal, the opening can be made large enough to facilitate the placement of the YSGG laser fiber such that it can effectively contact and irradiate all of the canal walls to achieve the optimal bactericidal effect.

Schoop *et al.* (2004)studied various laser systems namely the Nd:YAG, the diode, the Er:YAG, and the Er,Cr:YSGG laser, focusing on the respective wavelength, its specific bactericidal capabilities, and potential usefulness in root-canal disinfection with a suspension of either Escherichia coli or *Enterococcus faecalis* in the root canal extracted teeth. Microbiology test indicated that all laser systems were capable of significant reductions in both test strains. Using the higher setting of 1.5 W, significant reductions of *E. coli* were again observed with all laser systems. This study use Er,Cr:YSGG laser with output power of 1.0 and 1.5 W without any water spray and air cooling was significant different only in one log step to eradication *E.faecalis*.

The preliminary study by Eldeniz *et al.* (2007) using extracted teeth compared to 3 % NaOCl solution in contaminated root canal having small and large apical foramen. The use of Er,Cr:YSGG laser irradiation with output power of 0.5 W ,20 % air and water level did not eradicate all bacteria but 3 % NaOCl can inhibite all of *Enteroccus faecalis* and effective to sterile all root canals. The output power of the laser in this study is different from Schoop *et al.* (2004).

Wang *et al*. (2007) used an *in vitro* investigation to evaluate the bactericidal effect of the Er,Cr:YSGG laser and the Nd:YAG laser under standardized conditions compared with sodium hypochlorite (NaOCl) irrigation in experimentally infected root canals at an output power of either 1 W or 1.5 W. The Er,Cr:YSGG laser had a reduction of 77% after irradiation at 1 W and

96% at 1.5 W (no significant difference). The Nd:YAG laser had a reduction of 97% at 1 W and 98% at 1.5 W (no significant difference). The authors concluded that both lasers had a significant bactericidal effect, and the Nd:YAG laser was more effective. The study used Er,Cr:YSGG laser with output power 1.0 and 1.5 W without any water spray and air cooling similar to the study of Schoop *et al.* (2004).

Thus, if we use the maximum recommended power of this laser 1.5 W, without water and air cooling water whether the result of this study different from those of the former studies. Moreover there are rarely study to compare the antimicrobial effect of Er,Cr:YSGG laser with the standard irrigant solutions such as NaOCl or Chlorhexidine.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

1. Bacteria
   - *Enterococcus faecalis* ATCC29212
2. Nutrient
   - Mitis Salivarious agar
   - Tryptiene soy broth (Trypteine soy broth, Britania 30.0 g/L : Trypteine 17.0 g, Soy peptone 3.0g, Sodium chloride 5.0g, Dipotassium phosphate 2.5 g, Dextose 2.5g)
   - Tryptiene soy agar (Tripteine soy agar, Britania 40 g/L : Trypteine 15.0 g, Soy peptone 5.0 g, Sodium chloride 5.0 g, Agar 15.0 g)
3. Chemical solutions
   - 2.5% NaOCl (Lot. No. 56/51, Faculty of Dentistry, Chulalongkorn University)
   - 2% chlorhexidine solution (Lot. No.148 /51, Faculty of Dentistry, Chulalongkorn University)
   - 17% EDTA (Faculty of Dentistry, Chulalongkorn University)
   - Physiologic saline solution
   - Dye for Gram's stain
   - Distilled water
4. Instruments
   - Beaker 250, 1000 mL (Pyrex, Labware, USA)
   - Flask 100, 250, 1000 mL (Pyrex, Labware, USA)

- Micropipette (Eppendorf, Hamburg, Germany)
- Test tube
- Agar plate (Falcon, USA)
- Glass slide
- Glass beads
- Diamond fissure bur
- Gates Glidden drill No 2, 3, 4, 5
- K-file (Dentsply, Switzerland) No.10 -80
- H-file (Dentsply, Switzerland) No. 50
- Paper point size S, M, L
- Syringe size 5,10 ml
- Needle gauge 25
- Aluminum foil
- Nail polish
- Flowable composite resin (Z 350, 3MESPE,Thailand)

5. Equipments

- Autoclave (Tuttnauer 3370, USA)
- Incubator (Memmert, Germany)
- Laminar flow hood (Microflow advanced bio safety carbinet-class2, England)
- Spectrophotometer (Thermo spectronic genesys 20, USA)
- Colony Counter (Suntex, Taiwan)
- Vortex (Vortex-genic2)
- Microscope (Olympus CH-2, Japan)
- Er,Cr:YSGG laser and 200 μm endodontic fiber optic tip (Waterlase Millenium;Biolase Techn., San Clementa, CA,USA)
- Curing Light XL 3000 (3M Dental Product)

Figure 2 Incubator

Figure 3 Colony Counter

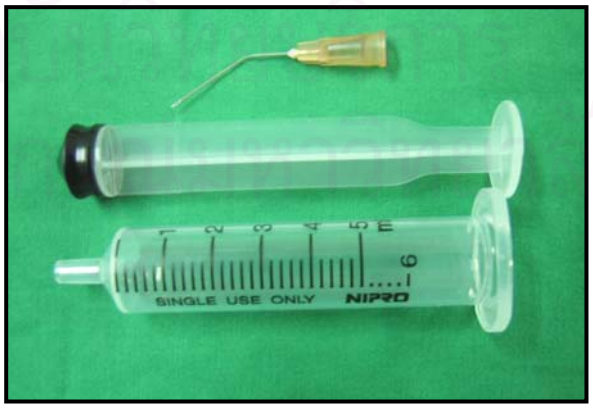

Figure 4 Syringe 5 ml and Needle gauge 25

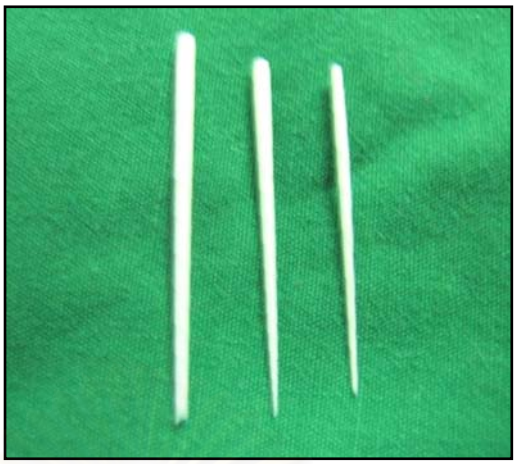

Figure 5 Paperpoint size L, M, S

3.2 Laser device

In this study, we use an Er,Cr:YSGG laser (Waterlase Millenium;Biolase Techn., San Clementa, CA, USA). This laser operates at a wavelength of 2,780 nm with a pulse energy that can be varied between 25 and 300 mJ at a fixed repetition rate of 20 Hz. This results an output power of 0.5-6 W. An automode will be used in this study, output power of 1.5 W, without water and air spray (Wang *et al*. 2007).The laser beam is delivered via a 200 μm endodontic fiberoptic tip.

Table 3 Laser parameters at an effective output of 1 and 1.5 W

Device	Wavelength (mm)	Pulse rate (Hz)	Pulse energy (mJ)(display)	Output power (W)(display)	Effective output Power (W)
Er,Cr:YSGG	2,780	20	not display	1.5 W	1
			not display	2.5 W	1.5

Figure 6 Er,Cr:YSGG laser

3.3 Sample preparation

One hundred and twenty-five extracted human single canal-rooted teeth were stored in saline solution until employed in the experiment. The teeth were prepared and instrumented using the following protocol:

I. The Coronal portion was removed at the cemento-enamel junction using diamond fissure bur with water coolant to obtain root canal length of 15 mm.

II. The pulp was removed and the working length of each root canal was established 1 mm short of the apical foramen with a K file size 20 (K-type file; Mani Inc., Nakaakutsu, Japan).

III. The coronal 1/3 of each canal was flared using Gates Glidden burs size 2, 3, 4 and debridement was completed with K-file to size 50 (K-type file; Mani Inc., Nakaakutsu, Japan) using crown-down technique. Sterile physiological saline was used as an irrigating solution after the completion of each file size.

IV. The apical foramen was closed with flowable composite resin (Z 350, 3MESPE,Thailand) then the root surface was sealed with two coats of nail polish.

The smear layer was removed by the sequential use of 5 ml of 17 % EDTA and 5.25 % NaOCl, for 3 minutes each. All teeth were individually placed in the plaster block approximately 6 mm tall for ease in handling and the orifices were closed with aluminium foil. The sterility of all root canals were achieved by autoclaving at 121 ^{o}C for 15 minutes.

To confirm sterility, 10 µl of the media was transferred into the canal lumen of every canal and left for 1 minute. The residual medium within the root canal was removed with sterile paper points, transferred to the test tube which contain 5 ml of TSB broth, vortex 1 minute and incubated 37 ^{o}C for 24 hours. Any sample showing turbidity was discard.

Figure 7. The sterility test of tooth canals prior to the inoculation demonstrating that all sample were sterile (no turbidity)

3.4 Bacteria inoculation (under laminar flow hood)

By Randomization, one hundred and twenty-five teeth were then divided to four groups of 30 teeth and 5 teeth were negative control group as follow.

Group 1 the sterility control group = 5 roots

Group 2 the neagative control group = 30 roots

Group 3 the 2.5% NaOCl group = 30 roots

Group 4 the 2% Chlorhexidine group = 30 roots

Group 5 the Er,Cr:YSGG laser irradiation group = 30 roots

Experiments were performed in set of 4-6 roots due to limitation of laser tips. Some procedures were repeated with remaining teeth until the proposed sample size.

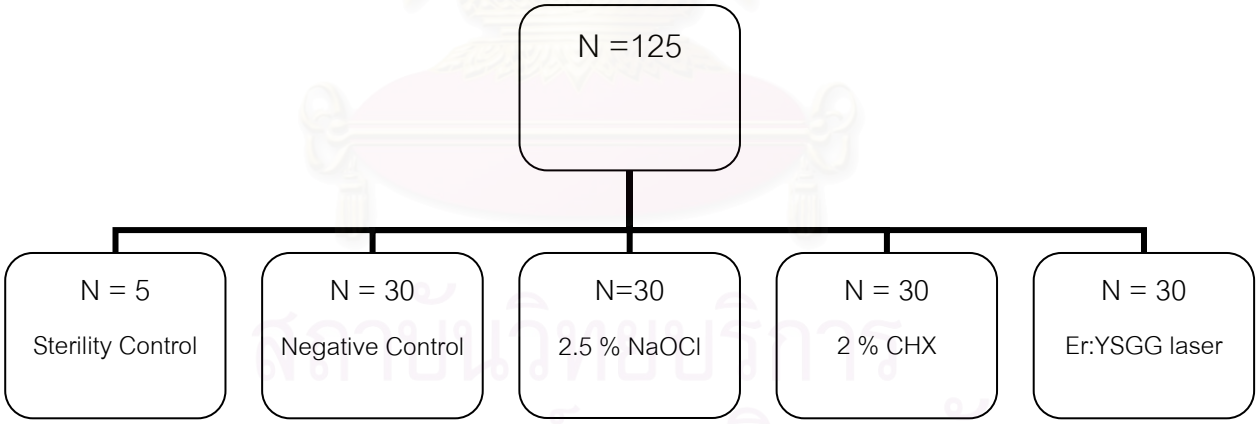

Figure 8 A diagram showing the randomized divided experimental groups

Enterococcus faecalis ATCC 29212 was used in this study. Before starting the experiments, the frozen (-20 ^{o}C) bacteria sample was thawed and grown for 24 h on a solid culture medium (MSA agar) at 37 ^{o}C under aerobic conditions. Five bacteria colonies were placed in TSB broth 25.0 ml and incubated for an additional 24 h at 37 ^{o}C under aerobic conditions. The purity of the strain was confirmed by Gram's stain. The cell suspension was adjusted to 10 ^{8} colony forming units per ml (CFU/ml) as determined by spectrophotometer with an OD 550 nm.

Ten µl of the bacteria culture were transferred into the canal lumen of the mechanically enlarged root canals using a sterile micropipette (Eppendorf, Hamburg, Germany) except the sterility control group and the orifices were closed with sterile aluminium foil. Then, these roots were incubated for 48 h at 37 ^{o}C.

Figure 9 A picture showing the tooth block in the plastic chamber before the incubation

3.5 Canal treatment

After 48 h, all canals weredried with sterile paper points.

- The first group was the sterility control group, the teeth were filled with 10 µl of fresh media as a control of the contamination and leakage of the sample during the experiment. Then the orifices were closed with sterile aluminium foil.

- The second group, the teeth were infected with *Enterococcus faecalis* and receiving no treatment to serve as the negative control group.

- The third group, each canal was irrigated with a 5.0 ml of 2.5 % NaOCl solution by delivering the solution 1 mm from the working length using sterile 5.0 ml plastic syringes and 25-gauge needles, left filled for 10 minutes and then final rinsed with 2 ml of distilled water.

- The fourth group, each canal was irrigated with a 5.0 ml of 2% CHX solution by delivering the solution 1 mm from the working length using sterile 5.0 ml plastic syringes and 25-gauge needle, left filled for 10 minutes for each canal and then final rinsed with 2 ml of distilled water.

- The last group, each canal was irradiated with the Er:YSGG laser (Waterlase Millennium; Biolase Tech., San Clemente, CA, USA), output power 1.5 W. The laser beam was delivered via a 200 µm endodontic fiberoptic tips. A fiber tip (Milennium; Biolase Technology Inc. (P/N 5000602), diameter of 200 µm was used. The fiber tip was inserted into the root canal at 1 mm from the working length. The laser was activated and the tip was slowly moved in a helicoidal manner from the apical to the cervical part of the canal for 10 seconds period with 15 seconds of rest between each lasing cycle. The total irradiation time was 40 seconds per canal.

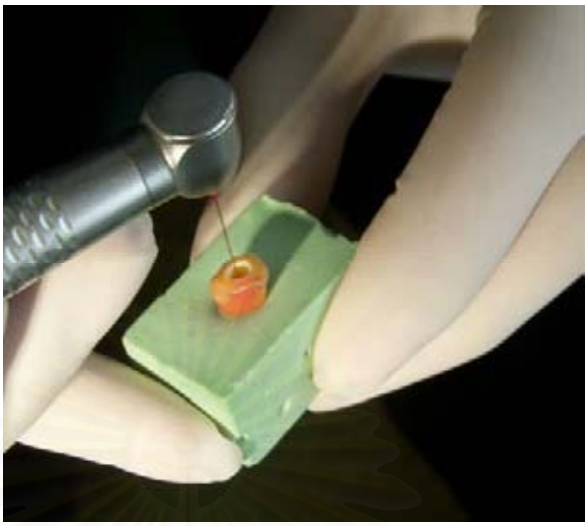

Figure 10. The irradiatIon of the Er:YSGG laser in the root canal

3.6 Bacterial analysis (under laminar flow hood)

After treatment, the liquid contents of root canals of all groups were carefully absorbed with sterile paper points without intentional touching the walls. All of the root canals were then filled with 10 µl sterile normal saline and gently circumferential filed with sterile H-file #50 at 1 mm short of the apex for 20 seconds. Next, the contents inside the canal were transferred using a sterile micropipette (Eppendorf, Hamburg, Germany) and immediately plated on TSA agar and incubated for 24 hr under standard aerobic conditions. In the negative control group, the contents were diluted a hundred folds before being plated on the agar. Colony forming unit (CFU) was counted. The purity of the strain would be confirmed again by Gram's stain.

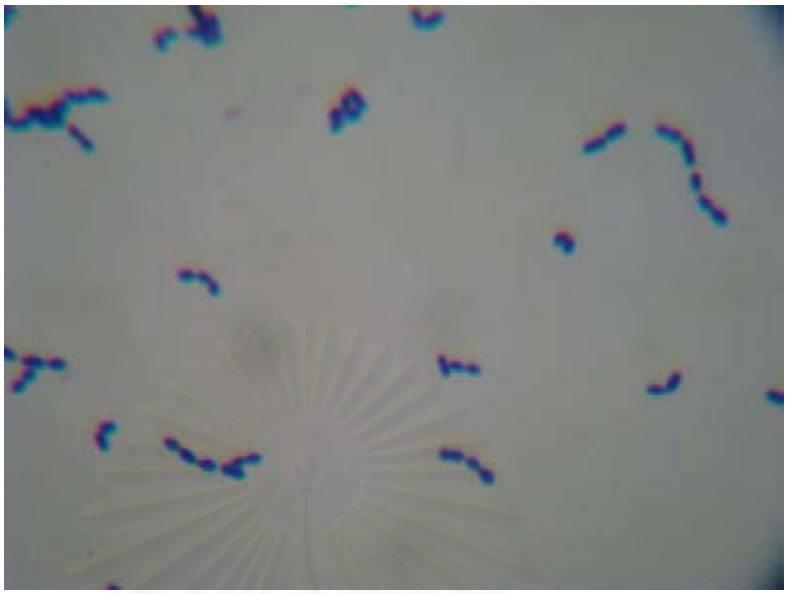

Figure 11 A picture showing the *Enterococcus faecalis* cell under a microscope (100X magnification, Olympus CH-2, Japan)

3.7 Statistic Analysis

The mean and standard deviation of CFU values were calculated. Statistical analysis performed using the SPSS program for Windows 15.0 (SPSS Inc., Chicago, IL, USA).

The original scale colony-forming unit (CFU) is not well summarized by an arithmetic mean. Therefore, the original CFU values were converted to Log CFU values, since the distribution of Log CFU data was well-modeled by normal distribution. The Log CFU data subjected to One-way ANOVA test for significant difference (α= 0.05) and the Tamhane Test is used for group comparison (α= 0.05).

CHAPTER IV

RESULTS

A total of 125 teeth were used in this study. Thirty teeth were assigned to each of 4 treatment groups. They were irrigated with 2.5%sodium hypochlorite (NaOCl); irrigated with 2% chlorhexidine (CHX); irradiated with Er,Cr:YSGG laser output 1.5 W; and no treatment as the negative control group. Five teeth were not inoculated and included as the sterility control group. Bacterial recovery was performed after treatment using the colony-forming unit (CFU) measurement in order to determine viable cells remaining in each sample.

According to the results from the study, the number of samples with no growth of bacteria observed are shown in Table 4.

Table 4 The number and percentage of samples with no growth of bacteria in each group.

Group	Number of samples with no growth of bacteria / total number of samples	Percentage
Negative Control	0/30	0%
NaOCl	23/30	76.7%
CHX	26/30	86.7%
Laser	0/30	0%

No growth of bacteria was observed in all samples in the sterility control group, whereas in the negative control group, the bacterial growth was recovered in all samples (30/30). Of all the treated groups, the CHX irrigation gave the highest number of sterilized teeth (26 out of 30 samples), as opposed to 23 out of 30 teeth in the NaOCl irrigation group and none (0 out of 30 teeth) in the laser irradiated group. When compared to the NaOCl and CHX groups with Fisher's Exact Test found that was no significant difference ($p > 0.05$) between group are demonstrated in Table 5.

Table 5 The comparison of the number of samples with no growth of bacteria between NaOCl and CHX groups using Fisher's Exact Test.

	Value	df	Asymp.Sig. (2-side)	Exact Sig. (2-side)	Exact Sig. (1-side)
Pearson Chi-Square	1.002[b]	1	.317		
Continuity Correction[a]	.445	1	.505		
Likelihood Ratio	1.012	1	.314		
Fisher's Exact Test				.506	.253
Linear-by-Linear Association	.985	1	.321		
N of Valid Case	60				

a. Computed only for a 2x2 table

b. 0 cell (0%) have expected count less than 5.The minimum expected count is 5.50

According to the results from this study, irradiation with Er,Cr:YSGG laser at the output power setting of 1.5 W failed to sterilize any of the 30 samples. Thus, it is concluded that NaOCl and CHX irrigation could eliminate bacteria better than the irradiation with Er,Cr.YSGG laser at the output power setting of 1.5 W.

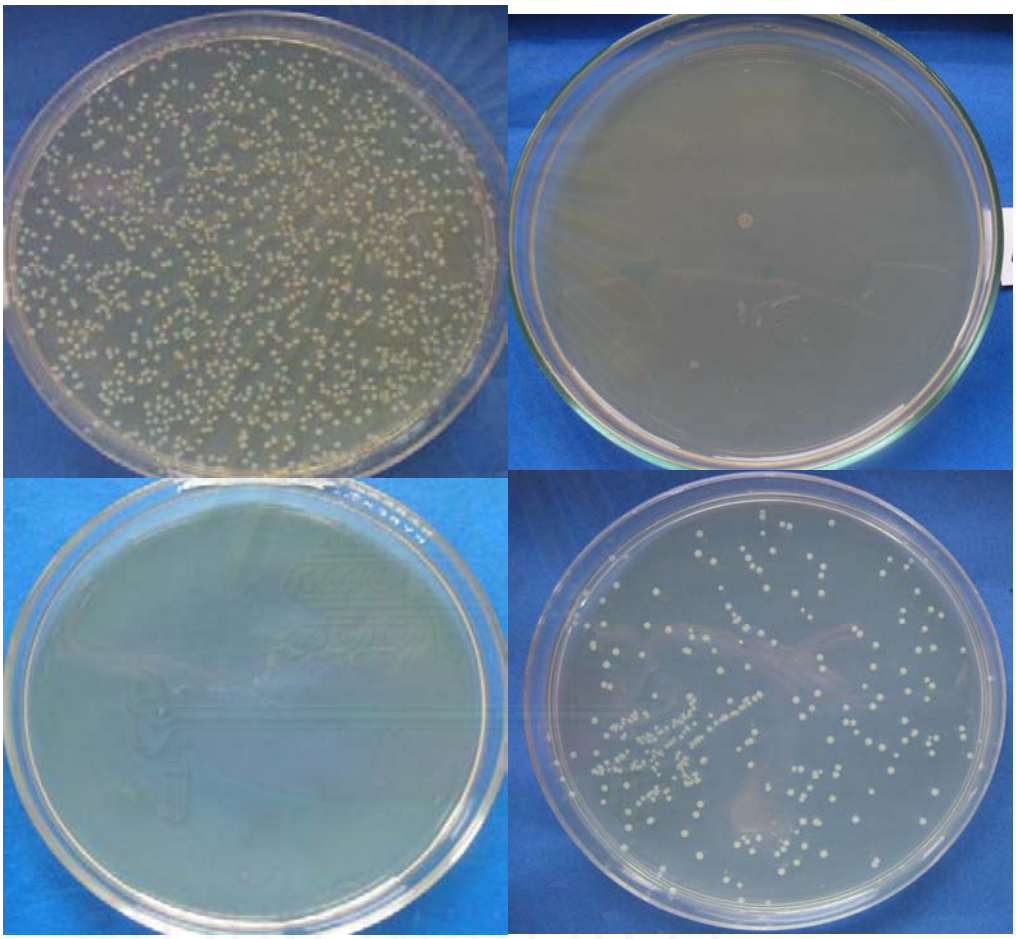

Figure12 A picture illustrating the result from the bacterial recovery experiment (CFU measurement) after the treatment. Bacterial colonies grown on TSA medium were observed in the negative control group at 1: 100 dilutions (Upper left) and the Laser group (Lower right). No bacterial growth observed in the NaOCl group (Upper right) and the CHX group (Lower left).

Table 6 The mean counts (CFU) of remaining bacteria after treatment in each group.

group	Mean (CFU)	Std. Deviation	N
Negative Control	53810.00	43342.090	30
NaOCl	1.71	1.113	7
CHX	1.50	1.00	4
Laser	280.40	227.969	30

The bacterial numbers (CFU) in each group are presented in Table 6. The high colony count of the negative control group (53810.00 ± 43342.090 CFU) suggested that bacteria survived the test period and confirmed the efficiency and confidence of the methodology used. Moreover it also demonstrated the viability of bacteria throughout inoculation, incubation processes, the further processing of the samples.

Among samples which still harbor bacteria after treatment. The low number of bacterial counts in the NaOCl and CHX group indicated that is these two treatment techniques were more effective compared to the treatment with Er,Cr:YSGG laser after which the bacterial count still remained considerably high.

The logarithm of CFU counts (Log CFU) was calculated in order to compare the number of bacteria of each group after the treatment. The average of Log CFU in each group are shown in Table 7 and the comparison among the four groups are also presented in Table 8.

Table 7 The mean Log CFU of remaining bacteria after treatment in each group.

Group	N	Minimum	Maximum	Mean	Std. Deviation
Negative Control (Log CFU)	30	9.13	11.82	10.4748	.99674
NaOCl (Log CFU)	7	.00	1.39	.3961	.54537
CHX (Log CFU)	4	.00	1.10	.2747	.54931
Laser (Log CFU)	30	3.83	6.74	5.3389	.77999

The negative control group had the highest mean Log CFU of number of microorganisms (10.4748 ± .99674) and there were statistically significant differences between the control group and the other groups ($p < 0.05$). Mean Log CFU values of NaOCl group and Chlohexidine group were 0.3961 ± 0.54537 CFU and 0.2747 ± 0.54931 CFU, respectively, but the difference was not statistically significant ($p > 0.05$). Mean Log CFU values after Er,Cr:YSGG laser irradiation was 5.3389 ± 0.77999 .This is statistically significantly higher than that of the NaOCl or chlorhexidine groups ($p < 0.05$).

Table 8 The comparison of Log CFU of remaining bacteria among the four treatment groups using Tamhane Test.

Multiple Comparisons

Dependent Variable : Log CFU

Tamhane

(I) group	(J) group	Mean Difference (I-J)
Negative Control	NaOCl	10.07869*
	CHX	10.20012*
	Laser	5.13585*
NaOCl	Negative Control	-10.07869*
	CHX	.12143
	Laser	-4.94283*
CHX	Negative Control	-10.20012*
	NaOCl	-.12143
	Laser	-5.06427*
Laser	Negative Control	-5.13585*
	NaOCl	4.94283*
	CHX	5.06427*

* The Mean difference is significant at the .05 level

The number of bacteria was statistically significantly reduced ($p<0.05$) by the two irrigating solutions and Er,Cr:YSGG laser compared with the control group. By comparing the Er,Cr:YSGG laser to the two irrigating solutions, the laser was statistically significantly less effective than irrigating with 2.5% NaOCl or 2 %CHX ($p<0.05$). There was no statistically significant difference between the two irrigating solutions ($p>0.05$).

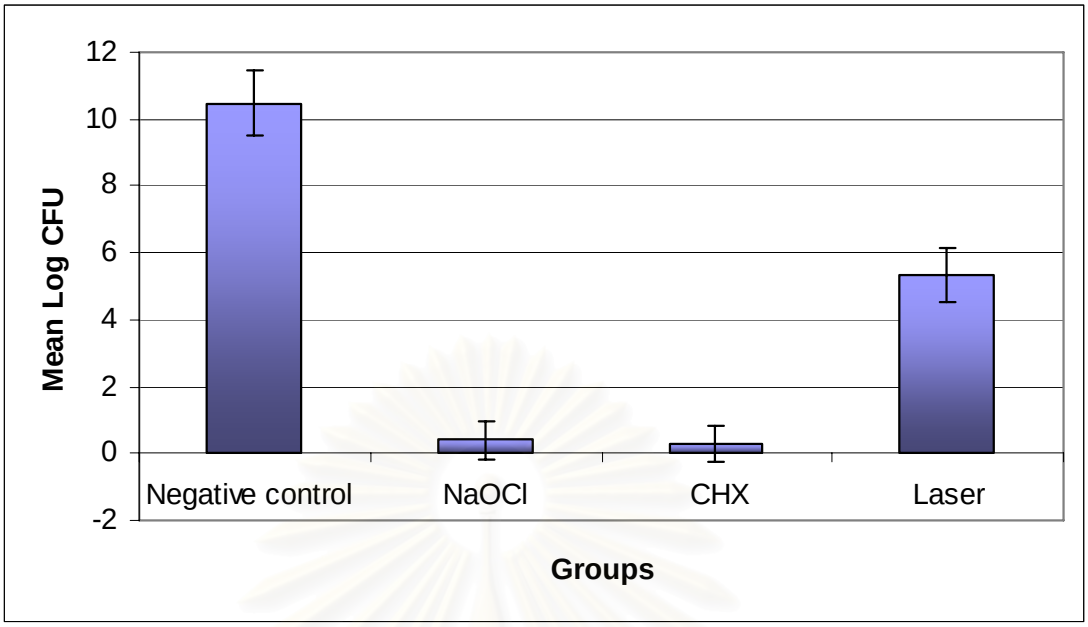

Figure 13 The comparison of the mean Log CFU in each group. A bar chart represents mean Log CFU of the remaining bacteria after treatment in each group. Error bars represent the standard deviation.

CHAPTER V

DISCUSSION AND CONCLUSION

Infections of the root canal system typically have a polymicrobial flora with approximately equal proportions of Gram negative and Gram positive bacteria (Nair *et al*., 1990; Sundqvist 1994). These bacteria permeate the three-dimensional tubular network of root dentin, and therefore, constitute an essential source for the potential reinfection of an endodontically treated root canal (Nair *et al*., 1990). Consequently, the complete removal of the pathogenic bacteria and their toxic byproducts is of crucial importance for the therapeutic outcome.

Conventional root canal treatment aims at the removal of the infected pulp and dentin layers by using mechanical and chemical techniques. However, these cleansing techniques are only successful to a certain extent.

Kouchi *et al*.(1980) demonstrated that bacteria are capable of invading the periluminal dentin up to a depth of 1,100 µm. On the other hand, chemical disinfectants penetrate no more than 130 µm into the dentin as indicated by Berutti *et al*. (1997) The discrepancy of the penetration depth between micro-organisms and bactericidal rinsing solutions often holds responsible for resistant cases and long-term failures which can be observed in conventional endodontics.

The purpose of this study was to compare the antimicrobial effect of Er,Cr:YSGG laser irradiation with the 2.5 % sodium hypochlorite and 2% chlorhexidine in extracted human teeth. In this study, a substantial number of single rooted teeth were collected and evaluated. As large numbers of teeth had an apical foramen size of 0.2 mm, this size was chosen as a typical apical foramen size. The root canal system was contaminated with *E. faecalis*.

Enterococcus faecalis is a facultative Gram-positive anaerobic coccus that is a known endodontic pathogen, being frequently recovered from the root canals of teeth associated with post-treatment diseases (Molander *et al*. 1998). Due to its high level of resistance to a wide range of antimicrobial agents including calcium hydroxide (Estrela *et al*., 1999; Haapasalo *et al*., 2000) and its ability to reside in canals as a single species without the support of other microorganisms (Fabricius *et al*,. 1982). It has been used in several previous studies of different disinfection methods (Orstavik *et al*., 1990; Ramskold *et al*., 1997; Schoop *et al*., 2002; Siqueira *et al*., 1997). It has also been previously used in several ex vivo studies to test the antimicrobial action of intracanal medication and lasers (Moritz *et al*., 2000; Orstavik & Haapasalo 1990; Schoop *et al*., 2004).

This study was designed to create a condition as similar as possible to *in vivo* situation. An *E.faecalis* suspension with 10^8 bacteria in 10 µl was used to inoculate the specimens' root canals. This corresponds to the maximum concentration of in vivo infected condition (Moritz *et al*., 1997). The methodology as perform by Eldeniz *et al*. (2007), Ramskold *et al.* (1997) and Le Goff *et al*. (1999) was used to contaminate the entire root canal system.

In the study, *E. faecalis* cells infected teeth were incubated for 48 hours in order to evaluate cells in the starvation phase rather than the growing cells. It was also reported that this microorganism has the ability under specific conditions to infect the whole length of the tubules within 2 days (Orstavik & Haapasalo 1990). This method was employed to better simulate conditions *in vivo* which a limited amount of nutrients would be available in the root canal (Portenier *et al*., 2005).

Sodium hypochlorite solution is the most commonly used root canal irrigant to date. However, no general agreement exists regarding its optimal

concentration, which ranges from 0.5% to 5.25%. Kozol *et al*. (1988) evaluated the toxic effects of NaOCl and observed that 0.025% was a safe concentration for clinical use as it maintains the antimicrobial action without harmful effects on the periapical tissues. NaOCl provides good tissue solvent action (Moorer *et al*., 1982) and has a broad spectrum of antimicrobial activity (Jeansonne *et al*. 1994; Siqueira & de Uzeda 1997), acts as a lubricant for instrumentation, and can flush loose debris from root canals (Baumgartner *et al*., 1992). The major disadvantages of NaOCl are its cytotoxic effect if injected into the periapical tissues (Spangberg *et al*. 1988), its foul smell and taste, its ability to bleach clothes, and its potential for causing corrosion (Busslinger *et al*., 1998). It is also known to produce allergic reactions (Kaufman *et al*., 1989). The disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution. HClO exerts its antimicrobial effect by an oxidative action on sulfhydryl groups of bacterial enzymes (Estrela 1999). As essential enzymes are inhibited, important metabolic reactions are disrupted and resulting in the killing of bacterial cells. However, some microorganisms such as *E. faecalis* are resistant to NaOCl at low concentrations (Baumgartner & Cuenin 1992; Gomes *et al*. 2001). On the other hand, the use of NaOCl at high concentrations is undesirable because it is an irritant to periapical tissues (Spangberg *et al*., 1988). Thus, in this study sodium hypochlorite with a concentration of 2.5 % was used.

The properties of CHX, such as broad spectrum of antimicrobial activity, substantivity, low toxicity, and water solubility, have increased the interest in its use in endodontics (Gomes *et al*., 2001). At low concentrations, CHX has a bacteriostatic effect. At higher concentrations, this agent has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking (Fardal *et al*., 1986). During root canal

preparation, the antimicrobial used should also act as lubricant, remove the smear layer, be water soluble, be biocompatible with periapical tissues, and have contact with the microorganisms. Two percentage chlorhexidine was evaluated in this study because it is more popular nowadays and there are few studies to compare its the antibacterial efficacy with the laser system.

The time period used for irrigation of the root canals in this study was 10 minutes which approximately corresponds approximately to the total time required for the biomechanical preparation of a root canal of moderate difficulty, Gomes *et al.* (2001) also used 10 minutes for irrigation in their study to test *in vitro* the effect of various concentrations of antimicrobial irrigants against *E. faecalis.* The microbes were killed in less than 30 seconds by the 5.25% solution, while it took 10 and 30 minutes for complete killing of the bacteria by 2.5% and 0.5% solutions, respectively. CHX digluconate, on the other hand, killed *E.faecalis* cells in 30 seconds or less in concentrations of 0.2-2 % (Oncag *et al*., 2003; Vianna *et al*., 2004).

The erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser is a laser system unit approved by the U.S.Food and Drug Administration for the cleansing, shaping and enlarging of the root canal (Rizoui *et al*., 1996;Yamazaki R *et al*., 2001). The Er,Cr:YSGG crystal generated photon through a fiber-optic cable delivery system terminating in a handpiece with sapphire crystal that is bathed in air-water spray (Rizoiu *et al*., 1996). The Er,Cr:YSGG laser emits an invisible beam in the infrared range of 2.79 micrometers, coupled with a nonabsorbing light source that serves as a pointer for the working laser (Goodis *et al*., 2002). The Er,Cr:YSGG laser is highly absorbed by water both surrounding and within the tissue. When laser energy is absorbed by the target, a reaction may occur depending on the total amount of energy applied, whilst the interaction type depends on power

density and pulse duration. A photo-thermal interaction with bacteria will result in a bactericidal effect. For this reason, it is possible that the laser may disinfect more efficiently in the absence of the water spray because this may focus more of the laser energy on the water within the bacterial cell. The Er,Cr:YSGG laser was used according to the manufacturer's instructions for sterilization and earlier descriptive procedure was strictly followed (Schoop *et al*., 2004). The output power setting of 1.5 W of laser was monitored before starting the experiment. According to the study by Wang *et al*. (2007), they found that the Er,Cr:YSGG laser had a bacterial reduction of 77% after irradiation at 1 W and 96% at 1.5 W with no significant difference. They used 4 cycles of 10 seconds with 15 seconds of rest between cycles (Wang *et al*., 2007). It has been suggested that too long lasing time may create a risk of thermal damage to dental tissues. The increase in the canal temperature and thermal transmission to the root surface can also cause damage to the surrounding periradicular tissue. The critical temperature for bone injuries lies at 47 ^{o}C, only 10 ^{o}C above normal body temperature (Ishizaki *et al*., 2004). The previous study of Schoop *et al*. (2004) found that temperature increased 8 ^{o}C after 1.5 W of Er,Cr:YSGG laser irradiation in the root canals which was in agreement with the study of Yamazaki *et al*. (2001).

In previous studies, residual bacteria after treatment were collected by many means: using paper points (Moshonov *et al*., 1995; Ramskold *et al*., 1997), rinsing (Fegan & Steiman 1995; Folwaczny *et al*., 2002; Hardee *et al*., 1994; Schoop *et al*., 2002), or immersion in culture broth (Zakariasen *et al*., 1986) or physiological saline solution (Moritz *et al*., 2000; Schoop *et al*., 2004). However, using those techniques, the volume of the sample recovered could not be quantified. In this study, canals were filled with sterile normal saline solution and then circumferentially filed for 20 seconds, after which the liquid

content in the canal was transferred onto TSA agar using a micropipette. According to Ørstavik & Haapasalo (1990), there is an overall good correlation between histology and culturing of dentine dust in their in vitro study. Peters *et al*. (2001) also confirmed that grinding and culturing of dentine gave better quantitative information about the extent of the infection. During the sampling procedure in this study, scraping of root canal wall were performed in order to obtain bacteria which may adhere to the wall or residing in the dentinal tubules.

This study evaluated the antimicrobial efficacy of the laser treatment by quantifying the residual number of *E. faecalis* (CFU) after treatment in infected root canals on TSA agar plates. The results showed that the Er,Cr;YSGG laser irradiation significantly reduced *E. faecalis* in vitro, although all samples showed some growth of bacteria. In 2.5% NaOCl and 2% chlohexidine irrigation groups, only a small number of bacteria could be found in the samples after treatment and highly significant reduction of *E. faecalis* when compared to the Er,Cr;YSGG laser irradiation group was demonstrated. The result is in agreement with previous studies which evaluated Er,Cr:YSGG laser. Jha *et al*. (2006) concluded that the Er,Cr:YSGG laser instrumentation was not able to eliminate *E. faecalis* infection in root canals and the laser was completely ineffective in disinfecting root canals. Eldeniz *et al.* (2007) founded that Er,Cr:YSGG laser irradiation did not eradicate all bacteria whereas 3 % NaOCl could inhibit all of *E. faecalis* and was effective to sterilize all root canals. They used 15 minutes irrigating time while was longer than that used in our study whereas the 0.5 W output power of laser was lower than that used in this study. In both of the studies, the investigators also recovered residual viable bacteria after laser treatment of infected root dentin by collecting dentin shavings from the root canal wall as conducted in this study.

The antibacterial effect of the laser was found to be less effective than 2.5% NaOCl solutions in this study. The results agree with former studies which compared various types of laser with NaOCl solution (Le Goff *et al*., 1999; Moshonov *et al*., 1995; Piccolomini *et al*., 2002). Le Goff *et al*. (1999) evaluated the bactericidal action of CO_2 laser and 3 % NaOCl on animal teeth infected with bacteria species. The CO_2 laser showed 85% decrease compared to the control group but NaOCl treatment was statistically superior to laser. Moreover, the comparative studies of NaOCl with Nd:YAG laser (Piccolomini *et al*., 2002) or argon laser (Moshonov *et al*., 1995) also gave the same results. However, the use of calcium hydroxide as an intracanal medicament is still recommended in a multiple-visit approach

In our model, the surviving bacteria were quantified by immediately collecting and plating the material recovered from the lased infected dentin. This allowed us to detect and measure the degree of disinfection achieved by the laser treatment. Although we did not find total elimination of viable organisms, we did achieve a significant reduction of the viable bacteria. The inability of the Er,Cr:YSGG laser in this study when used with fixed conditions (1.5 W, with no air and water level, 40 s) to completely kill the bacteria might be attributed to many reasons. First, with the available fiber optic, the laser beam can only deliver maximum energy to the area perpendicular to the tip where the beam is well focused, Hence, the use of a forward tip like this may not be able to perfectly direct the beam against the entire surface of the canal walls all the time. In this study, we tried to repeat a helicoidally movement of the tip from the apical to the cervical part several times during the lasing cycles in order to avoid the availability of the tip. Thus, one possibility to improve the bactericidal efficacy of the laser is the development of a new tip that can deliver the laser beam radially. Such newly designed laser tip, called

“radial fiber tip” will be available in the near future to offer predictable, total elimination of viable bacteria in the root canal wall (Schoop *et al.,* 2007 ; Gordon *et al.,* 2007). However, further studies are needed to verify its efficiency for clinical use in infected root canals. Secondly, prior smear layer removal might cause the bacteria to penetrate deeply into the dentinal tubules or some of them might reside in the ramification of the root canal system and then might be shielded from the laser beam. Other reasons might be the insensitivity of *E. faecalis* to laser irradiation because of its cell wall structure (Moritz *et al*., 2000) and/or the resistance of starved *E. faecalis* cells to different conditions (Portenier *et al*., 2005).

With the limitation of this research in mind, it can be concluded that Er,Cr:YSGG laser can reduce the *Enterococcus faecalis* in root canals to a certain extent but less effective than irrigating with 2.5 % sodium hypochlorite and 2% chlorhexidine solutions. However, at the present, the Er,Cr:YSGG laser could be considered as a supplement to the conventional protocols for the disinfection of the root canal system. Further improvements are still required to increase its antimicrobial efficacy.

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APPENDICES

Table 9 The number of *E. faecalis* at the optical density = 0.10

	รายละเอียด		
	Dilutian (100 ml)	ปริมาณ colony	CFU/ml
ครั้งที่ 1	10^4	1040	1.04×10^8
	10^5	110	1.10×10^8
	10^6	10	1×10^8
ครั้งที่ 2	10^4	1020	1.012×10^8
	10^5	109	1.09×10^8
	10^6	9	0.96×10^8
ครั้งที่ 3	10^4	998	0.99×10^8
	10^5	112	1.12×10^8
	10^6	10	1.2×10^8

The number of bacteria = 1×10^8 CFU/ml

Table 10 The number of remaining bacteria of the negative control group at 1 :100 dilutions in each sample.

No.of sample	The number of colony (1:100)	CFU
1	634	63400
2	884	88400
3	280	28000
4	223	22300
5	108	10800
6	120	12000
7	170	17000
8	98	9800
9	193	19300
10	132	13200
11	115	11500
12	92	9200
13	136	13600
14	99	9900
15	110	11000
16	100	10000
17	1004	100400
18	1352	135200
19	904	90400
20	608	60800
21	992	99200
22	364	36400
23	1128	112800
24	680	68000
25	923	92300
26	1052	105200
27	736	73600
28	1096	109600
29	448	44800
30	1362	136200

Table 11 The number of remaining bacteria of 2.5 % NaOCl group in each sample

No.of sample	The number of colony count	CFU
1	1	1
2	0	0
3	2	2
4	0	0
5	0	0
6	0	0
7	0	0
8	4	4
9	2	2
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	1	1
17	0	0
18	0	0
19	0	0
20	0	0
21	1	1
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0
28	1	1
29	0	0
30	0	0

Table 12 The number of bacteria of the 2% CHX group in each sample

No.of sample	The number of colony counts	CFU
1	0	0
2	0	0
3	1	1
4	1	1
5	0	0
6	0	0
7	0	0
8	1	1
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	3	3
25	0	0
26	0	0
27	0	0
28	0	0
29	0	0
30	0	0

Table 13 The number of remaining bacteria of the Er,Cr:YSGG laser group in each sample

NO.of sample	The number of colony counts	CFU
1	142	142
2	765	765
3	120	120
4	640	640
5	110	110
6	91	91
7	80	80
8	46	46
9	113	113
10	96	96
11	412	412
12	406	406
13	260	260
14	88	88
15	640	640
16	122	122
17	125	125
18	238	238
19	448	448
20	140	140
21	122	122
22	148	148
23	844	844
24	208	208
25	126	126
26	264	264
27	352	352
28	208	208
29	365	365
30	693	693

Table 14 Statistical analysis using SPSS 15 of Fisher's Exact Test between NaOCl and CHX groups.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CFU_R * group	60	100.0%	0	.0%	60	100.0%

CFU_R * group Crosstabulation

			group		Total
			NaOCl	CHX	NaOCl
CFU_R	0	Count	23	26	49
		% within CFU_R	46.9%	53.1%	100.0%
		% within group	76.7%	86.7%	81.7%
	1	Count	7	4	11
		% within CFU_R	63.6%	36.4%	100.0%
		% within group	23.3%	13.3%	18.3%
Total		Count	30	30	60
		% within CFU_R	50.0%	50.0%	100.0%
		% within group	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.002(b)	1	.317		
Continuity Correction(a)	.445	1	.505		
Likelihood Ratio	1.012	1	.314		
Fisher's Exact Test				.506	.253
Linear-by-Linear Association	.985	1	.321		
N of Valid Cases	60				

a Computed only for a 2x2 table

b 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.50.

Table 15 Statistical analysis using SPSS 15 of descriptive data of four treatment groups (CFU).

Descriptive Statistics

group		N	Minimum	Maximum	Mean	Std. Deviation
Control	CFU	30	9200	136200	53810.00	43342.090
	Valid N (listwise)	30				
NaOCl	CFU	7	1	4	1.71	1.113
	Valid N (listwise)	7				
CHX	CFU	4	1	3	1.50	1.000
	Valid N (listwise)	4				
Laser	CFU	30	46	844	280.40	227.969
	Valid N (listwise)	30				

Table 16 Statistical analysis using SPSS 15 of descriptive data of four treatment groups in Log CFU.

Descriptive Statistics

group		N	Minimum	Maximum	Mean	Std. Deviation
Control	LOGCFU	30	9.13	11.82	10.4748	.99674
	Valid N (listwise)	30				
NaOCl	LOGCFU	7	.00	1.39	.3961	.54537
	Valid N (listwise)	7				
CHX	LOGCFU	4	.00	1.10	.2747	.54931
	Valid N (listwise)	4				
Laser	LOGCFU	30	3.83	6.74	5.3389	.77999
	Valid N (listwise)	30				

Table 17 Statistical analysis using SPSS 15 of NPar Tests for normal distribution test each groups.

One-Sample Kolmogorov-Smirnov Test

group			LOGCFU
Control	N		30
	Normal Parameters(a,b)	Mean	10.4748
		Std. Deviation	.99674
	Most Extreme Differences	Absolute	.173
		Positive	.165
		Negative	-.173
	Kolmogorov-Smirnov Z		.947
	Asymp. Sig. (2-tailed)		.331
NaOCl	N		7
	Normal Parameters(a,b)	Mean	.3961
		Std. Deviation	.54537
	Most Extreme Differences	Absolute	.338
		Positive	.338
		Negative	-.234
	Kolmogorov-Smirnov Z		.893
	Asymp. Sig. (2-tailed)		.402
CHX	N		4
	Normal Parameters(a,b)	Mean	.2747
		Std. Deviation	.54931
	Most Extreme Differences	Absolute	.441
		Positive	.441
		Negative	-.309
	Kolmogorov-Smirnov Z		.883
	Asymp. Sig. (2-tailed)		.417
Laser	N		30
	Normal Parameters(a,b)	Mean	5.3389
		Std. Deviation	.77999
	Most Extreme Differences	Absolute	.169
		Positive	.169
		Negative	-.092
	Kolmogorov-Smirnov Z		.928
	Asymp. Sig. (2-tailed)		.356

a Test distribution is Normal. b Calculated from data.

Table 18 Statistical analysis using SPSS 15 of the comparison of Mean Log colony forming unit (Log CFU) among four treatment groups.

Test of Homogeneity of Variances

LOGCFU

Levene Statistic	df1	df2	Sig.
5.342	3	67	.002

ANOVA

LOGCFU

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	926.265	3	308.755	420.938	.000
Within Groups	49.144	67	.733		
Total	975.409	70			

Robust Tests of Equality of Means

LOGCFU

	Statistic(a)	df1	df2	Sig.
Brown-Forsythe	626.760	3	39.994	.000

a Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: LOGCFU

Tamhane

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	NaOCl	10.07869*	.27496	.000	9.2602	10.8972
	CHX	10.20012*	.32947	.000	8.9415	11.4588
	Laser	5.13585*	.23108	.000	4.5052	5.7665
NaOCl	Control	-10.07869*	.27496	.000	-10.8972	-9.2602
	CHX	.12143	.34340	1.000	-1.1700	1.4129
	Laser	-4.94283*	.25054	.000	-5.7238	-4.1618
CHX	Control	-10.20012*	.32947	.000	-11.4588	-8.9415
	NaOCl	-.12143	.34340	1.000	-1.4129	1.1700
	Laser	-5.06427*	.30938	.000	-6.3933	-3.7352
Laser	Control	-5.13585*	.23108	.000	-5.7665	-4.5052
	NaOCl	4.94283*	.25054	.000	4.1618	5.7238
	CHX	5.06427*	.30938	.000	3.7352	6.3933

*. The mean difference is significant at the .05 level.

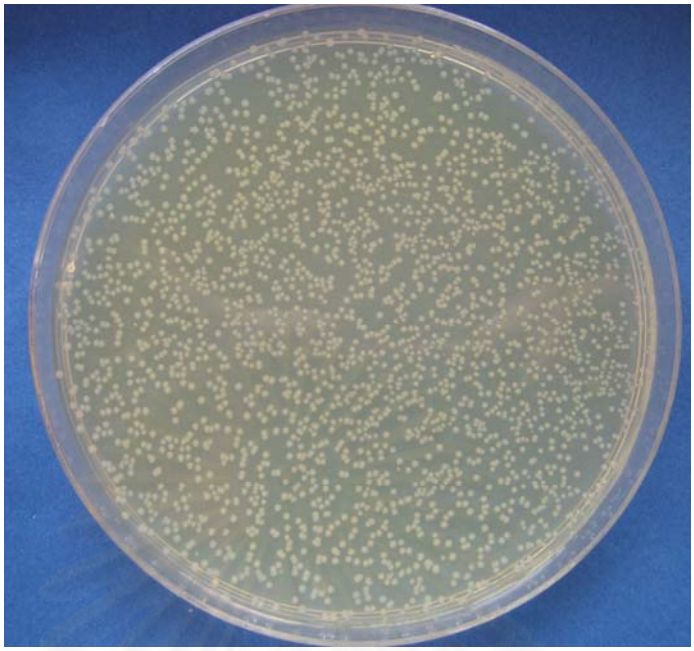

Figure 14 Illustration the colony growth on TSA of negative control group (1:10 dilutions).

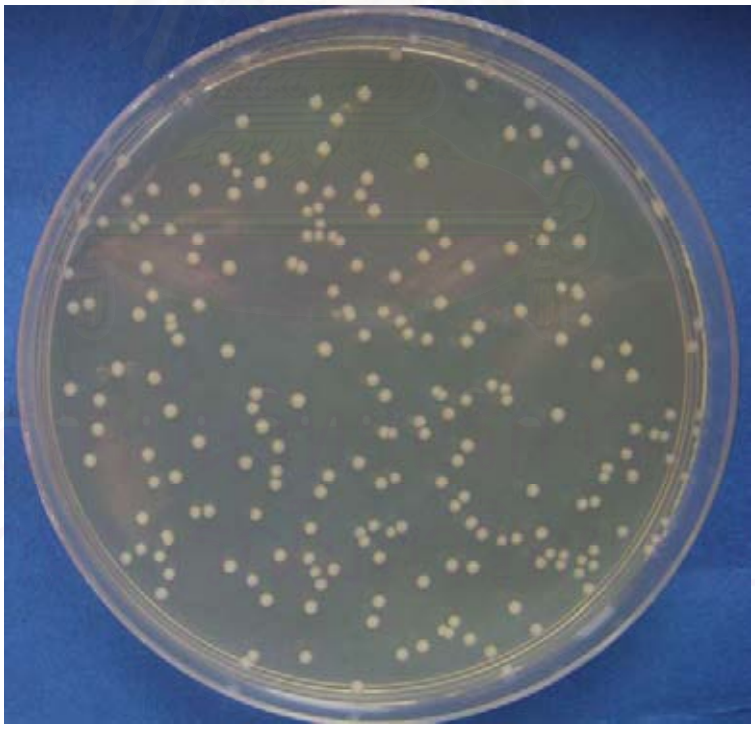

Figure 15 Illustration the colony growth on TSA of negative control group (1:100 dilutions).

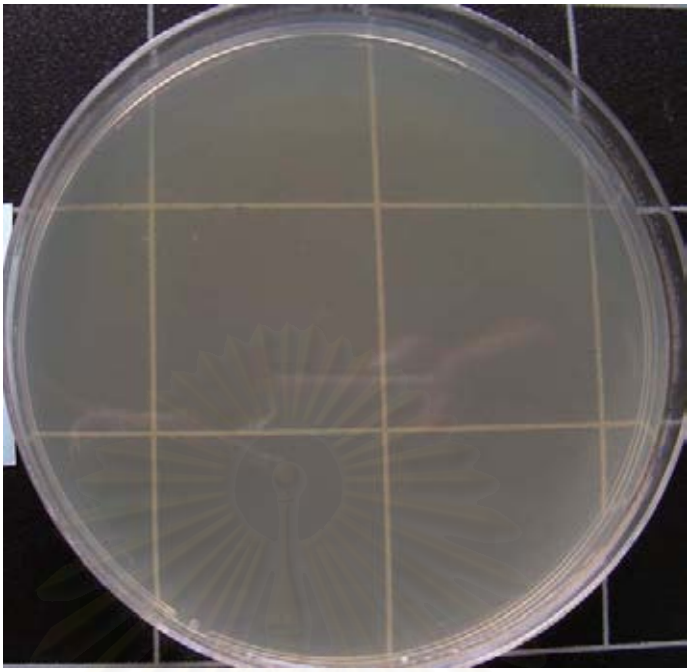

Figure 16 Illustration non of the colony growth on TSA of NaOCl group.

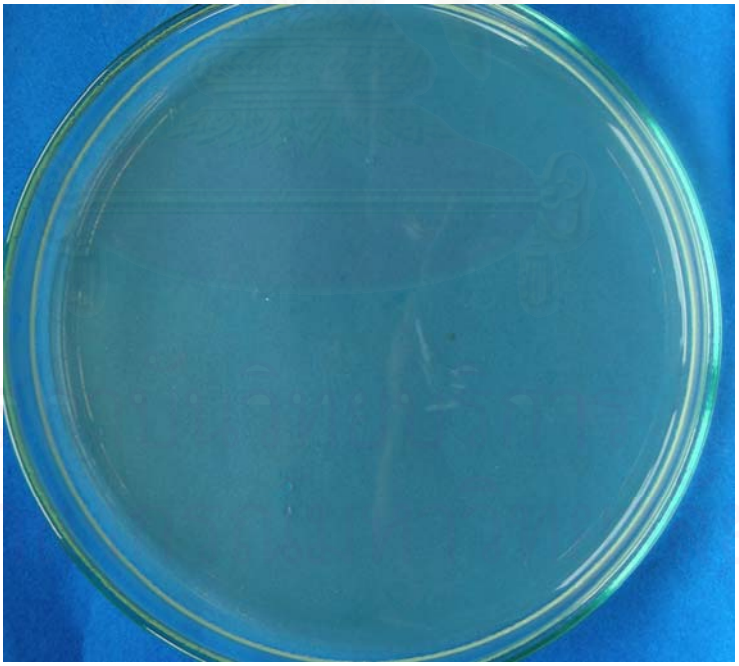

Figure 17 Illustration non of the colony growth on TSA of CHX group.

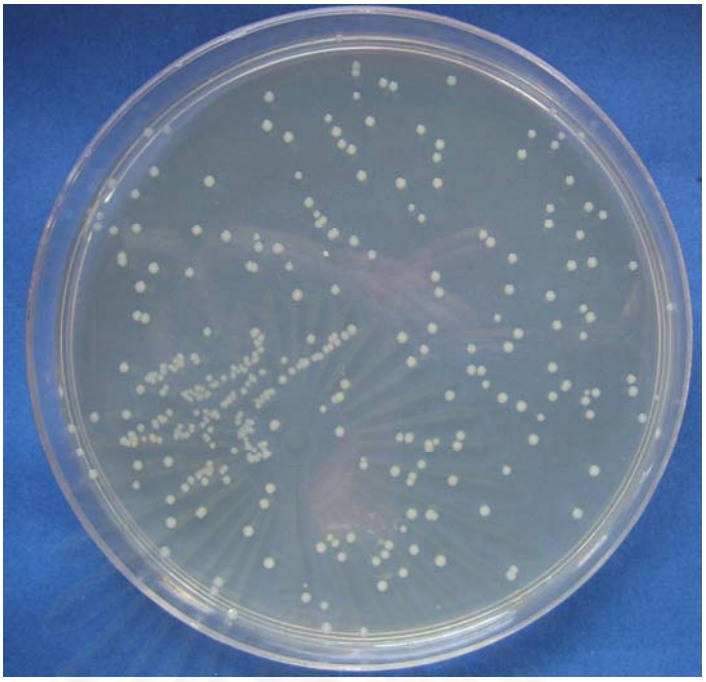

Figure 18 Illustration the colony growth on TSA of Er,Cr:YSGG laser group

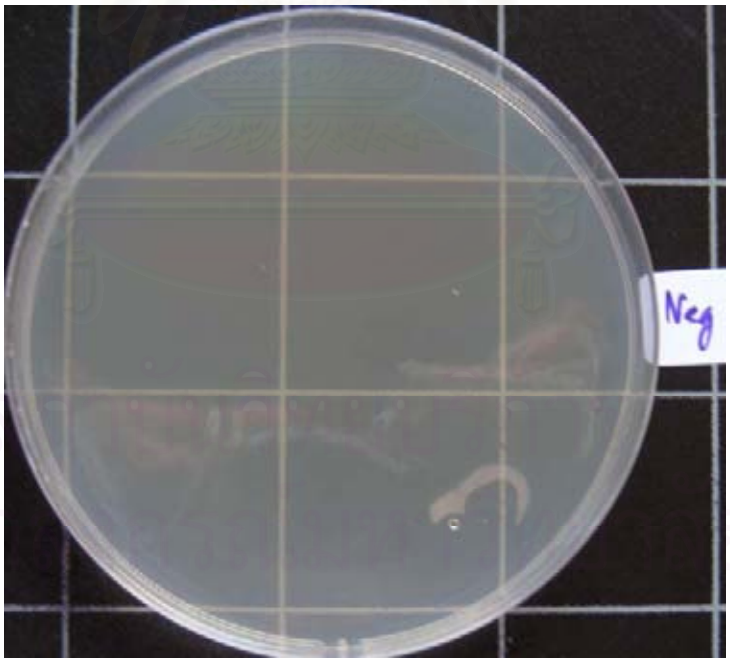

Figure 19 Illustration non of the colony growth on TSA of sterility control group.

BIOGRAPHY

Mr. thalerngsaks samaksamarn was born on 6 th of January 1973 in Surin. He graduated with D.D.S. (Doctor of Dental Surgery) from the Faculty of Dentistry, Chulalongkorn University in 1998, and became a staff member at Faculty of Dentistry, Khonkaen University. He studied in a Master degree program in Endodontology at Graduate School, Chulalongkorn University in 2007.