

Antibacterial Efficiency of Different Irrigation Solutions, Lasers and Photodynamic Therapy with Indocyanine Green in Root Canals Infected By *Enterococcus Faecalis*

Enterococcus Faecalis ile Enfekte Edilmiş Kök Kanallarında Farklı İrrigasyon Solüsyonlarının, Lazerlerin ve İndosiyanın Yeşili ile Fotodinamik Terapinin Antibakteriyel Etkinliği

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Abstract

Objective: The aim of this study was to evaluate bacterial reduction after using different irrigants, lasers and photodynamic therapy (PDT) in root canals infected by *Enterococcus faecalis*.

Materials and Methods: Seventy human maxillary incisors were used in this study. After endodontic preparation, sixty teeth were infected by 20 µl *E. faecalis* (ATCC 29212) and specimens were incubated for 72 hours. Groups were formed according to the irrigation protocol used: group 1: negative control, group 2: positive control, group 3: 10 mL 2% chlorhexidine (CHX), group 4: 10 mL 5% NaOCl, group 5: diode laser, group 6: Er:YAG laser, and group 7: indocyanine green (ICG)-diode laser (PDT). After disinfection procedures the samples isolated from root canals were placed into blood agar, incubated at 37 °C for 24 h and then were counted for colony-forming units. One-Way ANOVA was used for the statistical evaluation of data. For multiple comparisons, Tamhane's T2 test was used.

Results: All investigated disinfection procedures had a significant reduction in the bacterial population ($p<0.001$). CHX, NaOCl and ICG-diode laser groups were equally effective in reducing *E. faecalis* populations ($p>0.05$) and statistically more effective than both laser groups ($p<0.05$).

Conclusion: Promising results were obtained by using PDT with ICG. This alternative disinfection method found effective on *E. faecalis*.

Öz

Amaç: Bu çalışmanın amacı, *Enterococcus faecalis* ile enfekte edilmiş kök kanallarında farklı irrigasyon solüsyonları, lazerler ve fotodinamik tedavi (FDT) kullandıkten sonra bakteri sayısındaki azalmayı değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada yetmiş adet insan üst çene kesici diş kullanıldı. Kök kanal preparasyonundan sonra altmış diş 20 µL *E. faecalis* (ATCC 29212) ile

enfekte edildi ve numuneler 72 saat inkübasyona bırakıldı. Gruplar, kullanılan dezenfeksiyon protokolüne göre oluşturuldu: grup 1: Negatif kontrol, grup 2: Pozitif kontrol, grup 3: 10 mL %2 klorheksidin (KH), grup 4: 10 mL %5 NaOCl, grup 5: Diyon lazer, grup 6: Er:YAG lazer ve grup 7: İndosiyanın yeşili (İSY)-diyon lazer (FDT). Dezenfeksiyon prosedürlerinden sonra kök kanallarından izole edilen numuneler kanlı agara yerleştirildi, 37 °C'de 24 saat inkübe edildi ve daha sonra koloni oluşturan bireimler sayıldı. Verilerin istatistiksel değerlendirilmesi için tek yönlü varyans analizi, çoklu karşılaştırmalar için Tamhane'nin T2 testi kullanıldı.

Bulgular: İncelenen tüm dezenfeksiyon prosedürleri bakteri popülasyonunda belirgin bir azalmaya neden olmuştur ($p<0,001$). KH, NaOCl ve İSY-diyon lazer grupları, *E. faecalis* popülasyonlarının azaltılmasında eşit derecede ($p>0,05$), her iki lazer grubundan ise istatistiksel olarak anlamlı seviyede daha etkili ($p<0,05$).

Sonuç: İSY'nin kullanıldığı FDT'de umut verici sonuçlar elde edildi. Bu alternatif dezenfeksiyon yöntemi *E. faecalis* üzerinde etkili bulundu.

Introduction

The main objective of endodontic therapy is to eliminate pathogenic microorganisms which are the major confounding factor in the treatment of infected root canals and periapical healing (1).

Disinfection of dentinal tubules, accessory canals, isthmuses and ramifications that can be found in root canal system is not possible by mechanical preparation alone (2). Therefore, irrigation is one of the most important steps of endodontic treatment, which is recommended both before and during root canal shaping because of the antibacterial effects and necrotic tissue dissolving properties (3). The most commonly used irrigant in root canal therapy is sodium hypochlorite (NaOCl). Concentrations between 0.5% and 6% are preferred (4). Two-percent chlorhexidine (CHX) gluconate is an alternative endodontic solution (5).

The prevalence of endodontic infections depends on microorganisms' ability to adapt to environmental changes. Many different mechanisms are used by bacteria including biofilm formation, physiological modifications, exchange of genetic materials and formation of cell subpopulations (6). Despite the technological and scientific advances in endodontics, there are many unsuccessful cases due to microbial factors. *Enterococcus faecalis* is the most commonly isolated bacteria in teeth with apical periodontitis and is thought to be responsible for the majority of failed root canal treatments (7). *E. faecalis* may penetrate up to depths of 400-1000 µm in the dentin tubules, can live in conditions with poor nutrition and can develop resistance to root canal disinfectants (8).

Endodontic disinfectants have toxic and inadequate inorganic tissue dissolving properties, can not completely eliminate bacteria and cannot be tolerated by periapical tissues when extruded from

apex (9). These factors have prompted researchers to develop novel methods and materials for endodontic procedures (10-13).

Photodynamic therapy (PDT) shows promise as an antimicrobial therapy that can eliminate microorganisms observed in root canal infections. The PDT utilizes a non-toxic photosensitizing agent that has the capacity to generate highly reactive species, which are harmful for microorganisms and then irradiates through the application of the laser (14,15).

Recently used methylene blue and toluidine blue as photoactive agents were shown to cause discoloration of anterior teeth (12,16). Indocyanine green (ICG) is a photosensitizing agent that recently developed and used in many areas of medicine (17,18). Previous studies have reported that ICG did not lead to discoloration of teeth and did not produce any toxic or allergic effects. ICG displayed a strong adhesion to bacterial membranes (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Camphylobacter recta*, *Eikenella corrodens* and *Treponema denticola*) and demonstrated bactericidal effect when activated by a diode laser (19-21).

The aim of present study was to evaluate antibacterial effect of different irrigation solutions, two types of laser and PDT with ICG in infected root canals by *E. faecalis*. The null hypothesis tested was that these disinfection protocols had no influence on the eradication of *E. faecalis*.

Materials and Methods

The study was reviewed and approved by the Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (approval number: 14-KAEK-246). Seventy human maxillary incisors were used in this study. Mesio-distal and bucco-lingual direction radiographs

were taken from the teeth to confirm the presence of a single canal. Teeth were decoronated and 15 mm long roots were obtained, approximately 15 mm in length. Roots were prepared up to protaper F3 file (Dentsply-Maillefer, Ballaigues, Switzerland). After each file, 2 mL of 5% NaOCl (Whitedentmed, Erhan Kimya, İzmir, Turkey) was used. Five mL of 5% NaOCl, 5 mL of 15% ethylenediaminetetraacetic acid (Imicryl, Konya, Turkey) and 5 mL of 5% NaOCl solutions were used for the final irrigation respectively. Root canals were dried with paper points. Root apexes were sealed using composite resin. Specimens were placed into Eppendorf tubes with silicone impression material. Teeth were packaged one by one and sterilized in an autoclave at 121 °C for 30 minutes. After sterilization, 10 teeth were not infected to confirm the accuracy of the sterilization and were evaluated as a negative control group. *E. faecalis* cultures (ATCC 29212) were incubated for 72 hours and were placed in Brain Heart Infusion broth medium. Solution was prepared to be 0.5 Mc Farland. Each sample was inoculated with 20 µL of the prepared solution and specimens were incubated at 37 °C for 72 hours in the incubator. No disinfection procedure was applied to 10 samples, which were evaluated as the positive control group. In all experimental steps after sterilization samples were handled with sterile gloves, gauze and instruments in a laminar flow chamber to avoid contamination. Groups were formed as follows:

Group 1: Negative control (n=10). Specimens were sterilized and were not infected by *E. faecalis*.

Group 2: Positive control (n=10). Specimens were infected by *E. faecalis* but did not have a disinfection technique applied.

Group 3: CHX (n=10). Root canals were irrigated with 10 mL 2% CHX and 10 mL sterilized distilled water, respectively.

Group 4: NaOCl (n=10). Root canals were irrigated with 10 mL 5% NaOCl and 10 mL sterilized distilled water, respectively.

Group 5: Diode laser (n=10). Root canals were irradiated for 1 minute with a 940 nm wavelength diode laser (Epic, Biolase Tech., CA, USA) at 1W power utilizing the continuous mode with endodontic tips.

Group 6: Er:YAG (Erbium Yttrium Aluminium Garnet laser) (n=10). Root canals were irradiated for 1 minute with a 2940 nm wavelength Er:YAG laser (Kavo Key 3+, KaVo, Biberach, Germany) with endodontic

tips (diameter ISO 30). Laser Parameters were 1W, 10 Hz, and 100 mJ.

Group 7: ICG-diode laser (n=10). 25 mg sterile ICG mixed with 5 mL sterile distilled water in a sterile bottle. Each root canal was irrigated with 0.5 mL ICG solution then allowed to sit for 30 seconds. Then root canals were then irradiated for 1 minute (30 seconds wet, 30 seconds dry) with a 940 nm wavelength diode laser (Epic, Biolase Tech., CA, USA) at 1W power using the continuous mode setting with endodontic tips (diameter 200 µm).

Three sterile paper points for each tooth were moistened with 0.5 mL of sterile distilled water and were placed into the root canal one by one for 1 minute. Paper points were placed into sterile Eppendorf tubes with brain heart infusion medium and specimens were sent to the microbiology laboratory. Vortexing was performed on samples and 20 µL of liquid medium was inoculated on blood agar plates. Specimens were incubated at 37 °C for 24 hours and the colony-forming units (CFU) were counted at the end of incubation. Written consent wasn't obtained because operation was made on extracted teeth.

Statistical Analysis

One-Way ANOVA was used to evaluate the data. For multiple comparisons, Tamhane's T2 test was used. The significance was defined as p<0.05. Analyses were performed using SPSS 19.0 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY, USA).

Results

Results were summarized in Tables 1 and 2 and Figure 1. The highest bacterial colonization was observed in the positive control group. While there was no difference between negative control, NaOCl and the ICG-diode laser groups (p>0.05), there were statistical differences between the negative control and all other groups (p<0.05). There was no statistical difference between Er:YAG laser and diode laser groups (p>0.05). There were no statistically significant difference between CHX, NaOCl and PDT (ICG-diode laser) groups (p>0.05); bacterial colonization has been shown to be decreased when compared with laser groups used alone. Positive and negative bacterial growth three days after disinfection procedures were completed and are shown in Table 2. Laser groups did not completely eliminate *E. faecalis*, despite being quite effective in reducing bacterial colonization.

Table 1. Multiple comparisons between disinfection methods

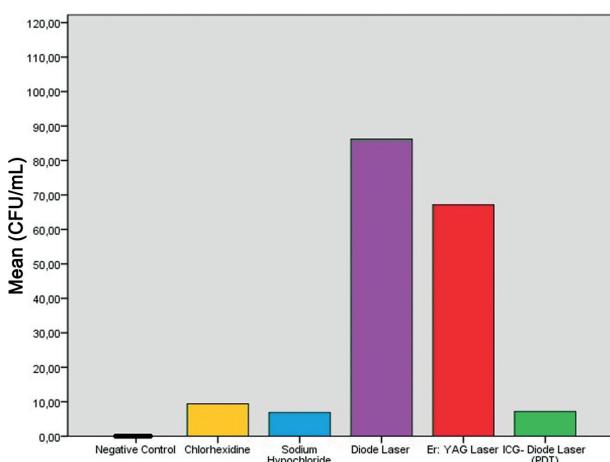
Groups	CFU/mL Mean ± SD	p
Negative control	0.00±0.00 ^A	<0.001
Positive control	8200±3794.73 ^D	
Chlorhexidine	9.40±7.07 ^B	
Sodium hypochlorite	6.90±15.37 ^{A,B}	
Diode laser	86.20±29.55 ^C	
Er:YAG laser	67.20±37.83 ^C	
ICG-diode laser (PDT)	7.20±10.16 ^{A,B}	

The results were considered statistically significant for p<0.05. For groups different uppercase letters (A,B,C,D) (One-Way ANOVA) indicate a statistically significant difference, SD: Standard deviation, CFU: Colony-forming units, ICG: Indocyanine green, PDT: Photodynamic therapy

Table 2. Bacterial growth after three days from disinfection of root canal system

Groups	Negative growth-count (n)	Positive growth-count (n)
Negative control	10	0
Positive control	0	10
Chlorhexidine	2	8
Sodium hypochlorite	7	3
Diode laser	0	10
Er:YAG laser	0	10
ICG-diode laser (PDT)	6	4

ICG: Indocyanine green, PDT: Photodynamic therapy

**Figure 1.** Graphic view of the results. The number of viable *E. faecalis* after each disinfection protocol was counted

CFU: Colony-forming units, ICG: Indocyanine green, PDT: Photodynamic therapy

Positive bacterial growth was observed in 10 samples from both the Er:YAG and diode laser groups. There were no bacterial growth in 7 samples in the NaOCl group, 6 samples in the ICG-diode laser group, and 2 samples from CHX group.

Discussion

Irrigation in addition to mechanical preparation is required in order to increase the efficiency of preparation and effectively eliminate bacteria from the root canal system (22). These irrigation solutions should eliminate endodontic pathogens, persistent infection and resistant bacteria. For this purpose, many solutions and their various concentrations and different disinfection methods are used in clinics and research (11,23-26).

However, the cytotoxic properties of the solutions beside antibacterial activity have prompted researchers to seek other options (27). Recently, new systems and materials have been proposed to replace the traditional chemomechanical process or supporting their impact to improve root canal disinfection (28). We aimed to demonstrate the effects of two types of lasers used alone and PDT with ICG on *E. faecalis* by comparison with conventional endodontic disinfectants. According to our results, NaOCl, ICG-diode laser group and CHX were more effective in eliminating *E. faecalis* than Er:YAG laser and diode laser disinfection methods. Therefore, the null hypothesis that the eradication of *E. faecalis* was not affected by the disinfection protocols was rejected.

Various studies have reported the antibacterial efficacy of PDT and have stated that PDT can be used safely without harming the host tissue (29-36).

Bonsor et al. (29) compared antimicrobial effect of PDT and 2.5% NaOCl on facultative anaerobic microorganisms on patients who were diagnosed irreversible pulpitis or periradicular periodontitis. Tolonium chloride as a photosensitizer and a diode laser as a light source were used. Researchers reported that 2.5% NaOCl provided an 80.9% bacterial reduction whereas PDT caused a bacterial elimination rate of 91.3% (29).

Fimple et al. (30) evaluated the effects of PDT application with methylene blue and a diode laser at the 665 nm wavelength in experimentally infected root canals by *Actinomyces israeli*, *F. nucleatum*,

P. gingivalis and *P. intermedia* and found an 80% bacterial decrease of CFUs. The findings of the study demonstrated that bacterial elimination could be achieved by more than $1 \log^{10}$ with an increasing concentration of methylene blue and light energy. Authors have concluded that PDT effectively helps the standard disinfection process with optimized parameters (30).

In another study that used conjugates of polyethylenimine and chlorine as a photosensitizer with a 660 nm wavelength diode laser with 40 mW energy as a light source, Garcez et al. (31) investigated the effects of PDT on antibiotic resistant microflora and retreatment of necrotic pulp teeth. Authors reported that using PDT after traditional chemomechanical preparation provided a microorganism-free root canal system and suggested the use of PDT in the presence of multi-drug-resistant organisms (31).

Pagonis et al. (32) evaluated the effects of PDT with nanoparticles loaded with methylene blue and a 665 nm wavelength diode laser on *E. faecalis* in the planktonic phase and reported that application of PDT led to a $1-2 \log^{10}$ bacterial reduction in CFUs (32).

Rios et al. (33) evaluated the antimicrobial effect of PDT with toluidine blue and a 638 nm wavelength light source on root canals infected by *E. faecalis*. They found that PDT resulted in a reduction of the bacterial count and suggested that PDT be used as a supportive antimicrobial process (33). The aforementioned study results are in agreement with this study results.

Nagayoshi et al. (34) studied the antimicrobial effects of PDT with ICG and an 805 nm wavelength diode laser at 5 W power in root canals infected by *E. faecalis* by using *in vitro* apical periodontitis models. The authors concluded that the diode laser irradiation combined with photosensitizer provided closer antimicrobial effect as obtained with 2.5% NaOCl and 60 seconds irradiation was more effective than 30 seconds (34). Similarly, Silva et al. (12) used a 660 nm wavelength diode laser at 40 mW power with methylene blue and malachite green as photosensitizing agents to evaluate disinfection of root canals by PDT. Authors suggested performing PDT with a diode laser and concluded that both photosensitizers for 60 and 120 seconds created more effective antibacterial effect against the *E. faecalis* than 30 seconds. (12). Therefore, in the present study, ICG solution was irradiated with a diode laser for 60

seconds in order to more closely simulate an actual clinical application period.

Souza et al. (35) examined the antimicrobial effects of PDT on *E. faecalis* by using methylene blue and toluidine blue as photosensitizers combined with NaOCl or sodium chloride and a 660 nm wavelength diode laser at 40 mW power as the light source. Authors concluded that PDT application in addition to traditional chemomechanical preparation and irrigation reduced populations of *E. faecalis* in root canals although the decrease was not statistically significant (35). These results are in contrast to the results of the present study. The difference in results may be attributed to the performed disinfection procedures and the used photosensitizer.

Juric et al. (36) investigated the antimicrobial efficacy of PDT in addition to retreatment. Researchers completed the endodontic therapy in a single session after using phenothiazine chloride photosensitizer and a 660 nm wavelength diode laser at 100 mW power in addition to chemomechanical preparation. Authors reported that PDT's use in combination with traditional chemomechanical root canal preparation led to significantly greater reductions of CFUs to indicate a decrease of the remaining bacteria or, in some cases, completely eliminated bacteria (36).

PDT was applied in dental practice in different wavelengths (between 488-906 nm) (14,37). A new 940 nm wavelength diode laser and ICG were used in the present study. As can be understood from our literature review, the role of PDT in the endodontic disinfection process had been tested with the use of the different photosensitizers, light sources and different laser parameters resulting in promising data indicating a reduction of *E. faecalis* in root canals. The results of the present study are consistent with what the researchers mentioned above found. PDT led to significantly higher bacterial reduction when compared with the diode laser group used alone and increased efficiency of the diode laser. However there was not a statistically significant difference in bacterial elimination when PDT was compared with traditional irrigants (5% NaOCl and 2% CHX).

Bago et al. (38) examined the antimicrobial activities of PDT and the traditional root canal disinfection techniques in *E. faecalis* infected root canals. For PDT applications, toluidine blue was used as a photosensitizer with a 660 nm wavelength diode

laser with 100 mW power was used in one group. Phenothiazine chloride as a photosensitizer and a 660 nm wavelength diode laser with 100 mW power was used in the other group. According to the findings of the study, PDT had the capacity to eradicate *E. faecalis* and is superior to traditional disinfection of 2.5% NaOCl (38). Although Bago et al. (38) found outstanding antibacterial activity following use of PDT when compared with NaOCl, their results partially coincide with present study results. In the present study, PDT was found to have the capacity to eliminate *E. faecalis* in serious rate but that PDT showed equivalent antibacterial activity as in NaOCl and CHX. We conclude that the reasons of this difference are time of application, different laser parameters and type of photosensitizing agent.

Tooth staining and discoloration are the main concerns of PDT, prompting the evaluation of the effectiveness of chemical components in an attempt to overcome this disadvantage. Carvalho Edos et al. (39) found an effective methodology to prevent tooth discoloration caused by methylene blue during PDT by using endo-PTC cream (10% urea peroxide, 15% tween 80 and 75% carbowax). The results of the present study showed that ICG did not lead to any visible tooth discoloration during the current application.

The *in vitro* model and using planktonic bacteria are the major limitations of this study. Unlike as in our model system, endodontic infections are typically polymicrobial by nature and have interactions between various microorganisms. Because of this, an *in vitro* culture may not reflect the *in vivo* condition and biofilm formation may affect results.

Conclusion

NaOCl is an irrigant that most commonly used in endodontics, easily available and inexpensive. Besides, NaOCl cannot be tolerated by periapical tissues and cytotoxic properties of NaOCl is disadvantage. Promising results were obtained by using PDT with ICG. ICG increased bacterial effectiveness of diode laser. There is need for further *in vivo* studies to support the *in vitro* promising results.

Ethics

Ethics Committee Approval: The study was reviewed and approved by the Ethics Committee of Gaziosmanpaşa University Faculty of Medicine (project number: 14-KAEK-246).

Informed Consent: Written consent wasn't obtained because operation was made on extracted teeth.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: İ.Ö., Material: İ.Ö., H.G., U.S.Ş.C., F.A., Design: İ.Ö., H.G., Data Collection or Processing: İ.Ö., H.G., U.S.Ş.C., F.A., Analysis or Interpretation: İ.Ö., H.G., U.S.Ş.C., F.A., Literature Search: İ.Ö., H.G., F.A., Writing: İ.Ö., H.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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