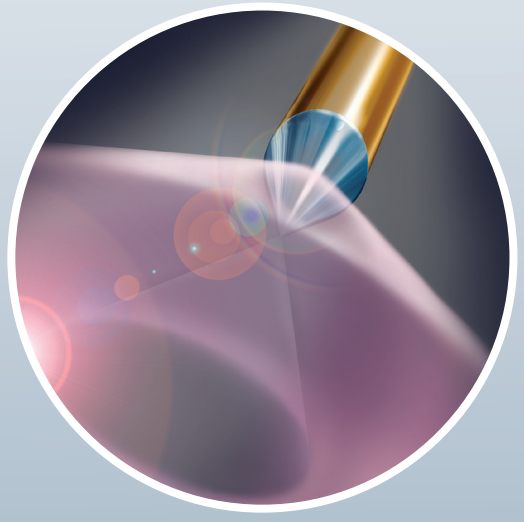

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The antimicrobial efficacy of the erbium, chromium:yttrium–scandium–gallium–garnet laser with radial emitting tips on root canal dentin walls infected with *Enterococcus faecalis*

Wanda Gordon, DMD; Vahid A. Atabakhsh, DDS; Fernando Meza, DMD; Aaron Doms, DDS; Roni Nissan, DMD; Ioana Rizoiu, MS; Roy H. Stevens, DDS, MS

ABSTRACT

Background. The authors used an in vitro model to investigate the ability of an erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser with radial emitting tips to disinfect *Enterococcus faecalis*–infected dentin.

Materials and Methods. The in vitro infected-dentin model system consisted of a dentin cylinder, prepared from a human anterior tooth root, cemented into a sealable two-chamber device fabricated from a syringe needle cap. The model's lower chamber contained a buffer solution, and the dentin cylinder was placed between the upper and lower chambers. After sterilization, the authors inoculated the root canal of each dentin cylinder with *E. faecalis*. They used an Er,Cr:YSGG laser with radial emitting tips to irradiate the root canal of each infected dentin cylinder (varying laser power and exposure time). After laser treatment, the authors machined the root canal dentin walls and collected the resulting dentin filings in the buffer-reservoir. They quantified the *E. faecalis* titer of each buffer-reservoir by using selective agar plates.

Results. The authors found that bacterial recovery decreased when laser irradiation duration or power increased. A greater degree of disinfection was achieved with a 120-second application of laser than with sodium hypochlorite treatment. Finally, they found that a 99.7 percent reduction in bacterial counts could be obtained using the laser.

Conclusion. The results of this study suggest that the Er,Cr:YSGG laser with a radial emitting tip has a significant antimicrobial effect on dentinal tubules infected with *E. faecalis*.

Clinical Implications. Er,Cr:YSGG laser treatment could be a valuable tool for root canal disinfection during endodontic treatment.

Keywords. Bacteria; disinfection; endodontic therapy; lasers; root canal.
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Bacteria are the primary causative agents in pulpal and periapical pathosis.[1,2] The challenge of nonsurgical endodontic treatment is to achieve total disinfection and elimination of bacteria from the root canal system. Clinical endodontic procedures rely on mechanical instrumentation and intracanal irrigants and medicaments to disinfect the root canal system. Although current instrumentation techniques involving hand and/or rotary instruments as well as ultrasonic and sonic devices can greatly reduce the bacterial load in the infected canal, they fall short of the goal of total disinfection of the root canal system.[3-5] Irrigants such as

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sodium hypochlorite and chlorhexidine also have demonstrated useful antimicrobial effects; however, here too, infection of the root canal and adjacent dentin many persist owing to the inability of these agents to reach all the infecting microorganisms.[6-9]

The use of intracanal medicaments such as calcium hydroxide typically requires multiple patient visits, since short-term application (of less than one week) has been found to be ineffective in eliminating endodontic infection.[10] The elimination of infection would seem to be a worthy goal, since research has shown that the absence of infection before obturation of a tooth undergoing endodontic treatment results in a higher success rate.[11] However, the multiple visits required for effective treatment with calcium hydroxide increases treatment time and reduces patient compliance, thus increasing the risk of treatment failure. Despite improvements in instrumentation techniques and the use of intracanal medicaments, endodontic treatment still can fail. Researchers have attributed such failure to the presence of residual intraradicular[12-14] or, less frequently, extraradicular[15,16] infection.

Enterococcus faecalis is a gram-positive facultative anaerobic bacterium, and it frequently is isolated from endodontic cases requiring retreatment.[17,18] It can infect dentinal tubules up to 800 micrometers from the root canal wall.[19] *E. faecalis* is resistant to calcium hydroxide treatment.[20-22] Sodium hypochlorite and chlorhexidine have proved to be effective against *E. faecalis* in vitro, but they require direct contact.[23,24] Consequently, clinicians should seek an alternative disinfection technique.

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 cleaning, shaping and enlarging the root canal as well as for use in osseous, apical and periodontal surgery. The Er,Cr:YSGG laser can remove calcified hard tissues by emitting a beam of infrared energy at 2.78 µm that works in combination with a water spray. Previous studies have examined the effects of this laser on mucocutaneous soft tissue and root canal walls.[25,26] The Er,Cr:YSGG laser is highly absorbed by water both surrounding and within the tissue. For this reason, it is possible that the laser may more efficiently disinfect tissue in the absence of a water spray, since this may focus more of the laser's energy on the water within the bacteria. In addition, radially emitting laser tips, the latest laser beam source (developed and manufactured by Biolase Technology, Irvine, Calif.), may prove to be an improvement for use in disinfecting root canals. Owing to the direction of their laser emission, these tips could provide better coverage of the root canal walls than conventional, forward-emitting tips. This effect could increase the probability that the emitted laser energy will enter the dentinal tubules and have an effect on bacteria that are some distance from the canal.

We conducted a study to evaluate the efficacy of the Er,Cr:YSGG laser (Waterlase, Biolase Technology) to disinfect *E. faecalis*–infected dentin when used with radially emitting laser tips.

MATERIALS AND METHODS

Model preparation. We sectioned 180 single-rooted teeth that had not been stored in sodium hypochlorite or any other disinfectant solution at the cementoenamel junction and at a point 3 to 4 millimeters from the apex. We then adjusted the resulting root section to a length of 5 mm. Using a diamond bur, we machined away the cementum and peripheral dentin of each root section, which resulted in a dentin cylinder approximately 5 mm in diameter that would fit within a hollowed-out plastic needle encasement (see below). Haapasalo and Orstavik[19] showed that removal of the cementum layer is important in facilitating the ingress of the infecting microorganisms into the dentinal tubules. We enlarged the canal of each root section with Peeso-type reamers sizes no. 1 through no. 4, resulting in a canal with an approximate volume of 8 microliters. To remove the smear layer, we then treated the sectioned roots with 17 percent ethylenediamine tetraacetic acid for four minutes, followed by 5.25 percent sodium hypochlorite for another four minutes, and finally rinsed the roots in sterile water for 30 minutes.

We infected the dentin cylinder models for one week, according to the protocol described by Haapassalo and Orstavik.[19] We modified a 30-gauge

ABBREVIATION KEY. BHI: Brain-heart infusion. **BSG:** Buffered saline with gelatin. **CFU:** Colony-forming unit. **Er,Cr:YSGG:** Erbium, chromium:yttrium-scandium-gallium-garnet. **Log CFU:** Logarithmic scale colony-forming unit. **TA:** Thallous acetate.

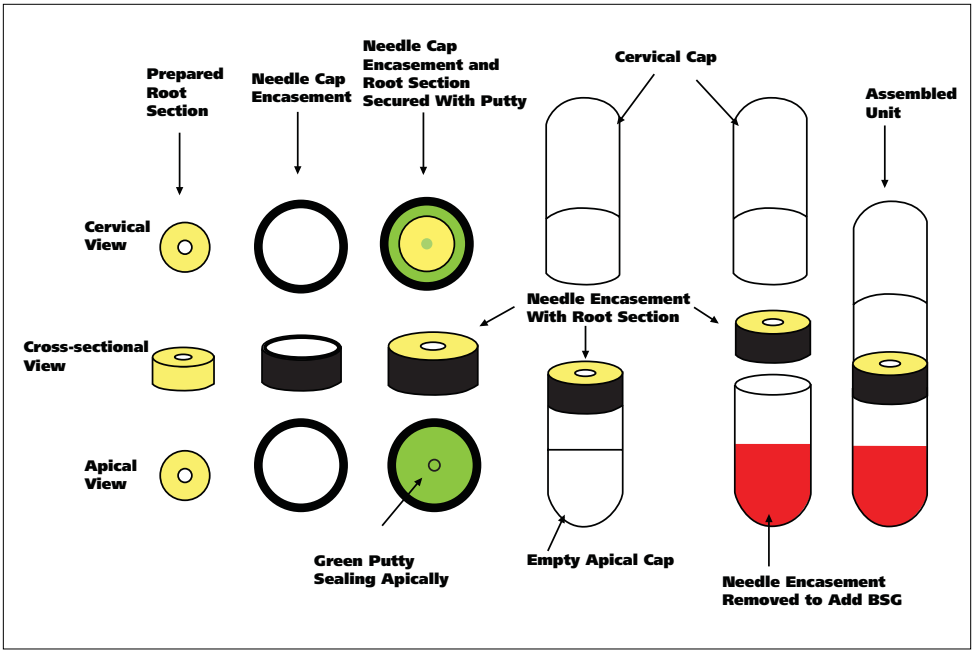

Figure 1. Model preparation. Buffered saline with gelatin (BSG) contains 8.5 grams sodium chloride, 0.3 g anhydrous monopotassium phosphate, 0.6 g anhydrous disodium phosphate and 0.1 g of gelatin per liter of distilled water.

Figure 2. Assembled and disassembled dentin cylinder model.

blue syringe needle assembly by uncapping and removing the needle from its encasement, then hollowing out the encasement with a bur. We applied Kneadatite epoxy putty (Polymeric Systems, Phoenixville, Pa.) (certified at 300 F and 2,000 pounds per square inch) to the external surface of each root section as well as to the apical end of each root section to create an airtight seal between the external dentin surface and plastic when the root section was placed in the hollowed-out needle encasement. The root section thereby was secured in place with the putty. We pipetted 500 µL of buffered saline with gelatin solution (BSG: sodium chloride 0.85 percent, anhydrous monopotassium phosphate 0.03 percent, anhydrous disodium phosphate 0.06 percent and gelatin 0.01 percent) into the apical cap, thus forming a buffer reservoir. We then returned the encasement (containing the root section) to the apical cap, over which we placed the cervical cap.

The putty in all the models was allowed to set for at least 12 hours. This model allowed for a cap to cover both the cervical and apical ends of the root sections individually, thus creating a closed system (Figures 1 and 2). We stored the models in a cold, damp location to prevent the teeth from drying out. We autoclaved the models with slow exhaust for 15 minutes at 120 C before infection. During sterilization, approximately 50 µL of BSG in the reservoir was lost owing to evaporation, leaving 450 µL of BSG in the lower reservoir of the model. We divided the models into groups 1 through 18, with 10 models in each group, as shown in Table 1.

Incubation conditions. We prepared overnight broth cultures (grown at 37 C) of *E. faecalis* (American Type Culture Collection 29212) in brain-heart infusion (BHI) broth (Difco Laboratories, Sparks, Md.). We used the broth cultures to infect the root dentin models.

Infection of models. We used a micropipette with a sterile, gel-loading tip to transfer 8 µL of an overnight BHI broth culture of *E. faecalis* 29212 to the canal space of each model. We performed this inoculation by removing the cervical cap of each assembled model, injecting the bacterial sample into the canal space and replacing the cervical cap. After inoculation, we incubated all

models in a moist environment in a warm room (37 C) for seven days. We found this incubation period to be sufficient for adequate infection of the dentinal tubules on the basis of a study by Haapasalo and Orstavik,[19] who found that *E. faecalis* would not grow significantly further into dentinal tubules of the root dentin if incubation was continued beyond seven days. We used sterile micropipettes to add 4 µL of BHI to the root canal of each root section once daily throughout the incubation period to prevent desiccation and provide nutrients for the bacteria within the tubules to grow.

Treatment of canals. After the seven-day incubation period, we dried the canals in all the models with sterile paper points. We treated groups 1, 2 and 3 (the control groups) as follows:

- we provided group 1 models with no disinfection treatment;
- we irrigated group 2 models with 1.5 mL of 2.5 percent sodium hypochlorite and then dried them;
- we irrigated group 3 models with 3.0 mL of 2.5 percent sodium hypochlorite and then dried them.

We lased the models from groups 4 through 18 using the Er,Cr:YSGG radially emitting laser tips inside the models' canals. We used sterile gloves, a sterilized laser handpiece, sterilized water and sterilized Z2 type (200 µm diameter and 14 mm in length) radially emitting laser instrument tips for each sample. For the groups that we lased in the presence of an air-water spray (groups 4, 7, 10, 13 and 16), we carried out the lasing with the Er,Cr:YSGG laser unit set to emit 175 ± 25 milliwatts power output variation limit at 20 hertz, with 34 percent air and 28 percent water. We moved the radially emitting laser tip by hand up and down in the canal in a cervical-apical and apical-cervical direction at a rate of 1 mm per second (that is, 10 seconds to traverse the full 5-mm length of the canal in both directions). During this procedure, we kept the tip as close to the canal wall as possible.

For the groups lased without water, we set the laser power at either at 175 ± 25 mW power and 20 Hz (groups 5, 8, 11, 14 and 17) or at 350 ± 50 mW and 20 Hz (for groups 6, 9, 12, 15 and 18). The indicated laser emission settings (175 ± 25 mW and 350 ± 50 mW) take into consideration a variation of the measured power output of no more than 15 percent and a 68 percent attenuation of power that occurs as a result of using a 200-µm fiber tip. Therefore, the laser settings are representative of the actual power output calculated for the 200-µm fiber tips, not the display power shown on the laser unit. Each fiber tip was used to treat 10 models—that is, all the models in one experimental group. The measured power loss after 10 treatments was between 10 and 15 percent.

TABLE 1

Group definitions.

GROUP NUMBER	TREATMENT
Control Groups	
1	Not treated (positive control)
2	Irrigated with 1.5 milliliters of 2.5 percent sodium hypochlorite
3	Irrigated with 3.0 mL of 2.5 percent sodium hypochlorite
Lased Groups	
4	15 seconds, 175 milliwatts + air + water spray*
5	15 seconds, 175 mW dry†
6	15 seconds, 350 mW dry
7	30 seconds, 175 mW + air + water spray
8	30 seconds, 175 mW dry
9	30 seconds, 350 mW dry
10	60 seconds, 175 mW + air + water spray
11	60 seconds, 175 mW dry
12	60 seconds, 350 mW dry
13	120 seconds, 175 mW + air + water spray
14	120 seconds, 175 mW dry
15	120 seconds, 350 mW dry
16	240 seconds, 175 mW + air + water spray
17	240 seconds, 175 mW dry
18	240 seconds, 350 mW dry

* Air 34 percent, water 28 percent.
† 0 percent air and water.

Recovery and quantification of *E. faecalis* from infected canals. Immediately after treatment, we uncapped all models of all the groups (1-18), dried the canals with paper points and then enlarged the canals with sterilized no. 5 and no. 6 Gates-Glidden burs through each orifice and the apical putty to allow dentinal filings to fall into the lower reservoir filled with 450 µL of BSG. (In a previous pilot study, we found the use of Gates-Glidden burs to be an effective method of recovering infected dentinal filings in the buffer reservoir [R.H. Stevens, unpublished data, October 2004].) We then used 50 µL of BSG to wash down remaining filings through the apical orifice to make a combined total of 500 µL of undiluted sample in the BSG reservoir. We conducted the drilling under aseptic conditions. We vortexed the undiluted samples and then allowed them to sit

undisturbed for a few minutes before we prepared serial dilutions. The undiluted samples (containing the infected dentin filings) were diluted serially (10-fold dilutions) from 10^{-1} to 10^{-3} with BSG. We spread 50-µL aliquots from each dilution on modified thallous acetate (TA) agar plates (proteose peptone 1 percent, yeast extract 1 percent, glucose 1 percent, TA 0.2 percent, triphenyl tetrazolium chloride 0.01 percent, agar 1.3 percent), which is selective for enterococci.[27] We then incubated the plates overnight at 37 C, and we counted the resulting colonies.

Statistical methods. The original scale colony-forming unit (CFU) has a highly skewed distribution that is not well-summarized by an arithmetic mean. Therefore, we converted all original CFU values to a base 10 logarithmic scale (log CFU), since the distribution of the log CFU data was well-modeled by a normal (Gaussian) distribution. We then converted the base 10 log means to geometric means on the original scale by calculating the base 10 antilog of the log mean CFU value. We also computed the base 10 antilog of the log scale 95 percent confidence intervals to determine the original scale 95 percent confidence intervals.

We used one-way analysis of variance methods to compare means on the log scale, including comparisons with controls. Using linear regression, we assessed the influence of time and the three laser conditions (low versus high wattage, wet versus dry technique) and their potential interactions on log CFU.

We chose *Enterococcus faecalis* as the test microorganism in this study owing to its high frequency of isolation from cases of failed endodontic treatment, its resistance to calcium hydroxide treatment and its relative insensitivity to laser irradiation.

RESULTS

Table 2 summarizes mean log CFU and CFU values for all 18 groups, along with the lower and upper boundaries of the 95 percent confidence interval for the geometric mean CFU.

Figure 3 (page 998) shows a plot showing mean log (base 10) CFU values with time for the three laser treatment conditions: $P = 175 \pm 25$ mW at 20 Hz with spray (wet), $P = 175 \pm 25$ mW at 20 Hz without spray (dry) and $P = 350 \pm 50$ mW at 20 Hz without spray (dry). Figure 3 shows that, of the three different laser treatment conditions, the $P = 350 \pm 50$ mW at 20 Hz without spray (high-wattage dry) had the lowest recoverable mean log CFU for four of five laser-exposure time settings. The exposure times for the laser treatments represent the summation of discrete exposures of five seconds each. Therefore, for a cumulative exposure of 30 seconds the laser was fired six times, five seconds each time, and the fiber tip was moved at a rate of 1 mm per second either upward or downward within the root canal.

Table 3 (page 998) summarizes the mean difference (base 10 log scale) of CFU, the percentage reduction of CFU and P values for groups 2 through 18 relative to nontreated control (group 1). Statistically significant differences (P values $\leq .05$) exist for all groups relative to control group 1. The smaller the mean CFU as a percentage of the control mean, the larger the percentage reduction in CFUs. For example, the lowest percentage of CFUs, 0.29 percent, which we calculated for group 18 ($P = 350 \pm 50$ mW dry mode), corresponds to a 99.7 percentage reduction of the mean CFU determined for control group 1.

Table 4 (page 999) shows the results from the regression of log CFU on time for the three wattage and wet-versus-dry conditions. From the 180 observations (18 groups, 10 observations per group), we used 147 cases in the regression model. We did not include the 30 observations of groups 1, 2 and 3 because they represent the nonlaser study conditions. The other three excluded observations were related to samples that had leaked during testing. The model showed that the time by wattage-wet/dry condition interaction was not statistically significant ($P = .10$), implying parallel behavior over time. However, the mean effects of all three predictors, time, wattage and wet/dry technique were significant at $\alpha = .05$.

The pooled data regression model posits that, on average, log CFU decreased by 0.0021 log units per second overall and was highest under low-wattage wet conditions, next highest under low-wattage dry conditions and lowest under high-wattage dry conditions. When we controlled for time, we found that the low-wattage dry condition had log CFUs that were 0.25 log units lower than the low-wattage wet condition on average

TABLE 2

Summary of statistical data.*

GROUP	TREATMENT	MEAN LOG CFU*	SE†	LB 95% CI‡	GEOMETRIC MEAN CFU§	UB 95% CI¶
1	None (positive control)	4.67	0.19	19,258.93	46,397.03	111,775.89
2	Sodium hypochlorite 1.5 milliliters	2.96	0.19	376.55	907.16	2,185.45
3	Sodium hypochlorite 3.0 mL	2.58	0.23	133.95	383.13	1,095.86
4	15 seconds P = 175 milliwatts with spray	3.39	0.19	1,020.13	2,457.62	5,920.71
5	15 seconds P = 175 mW dry	3.04	0.19	457.36	1,101.82	2,654.42
6	15 seconds P = 350 mW dry	2.69	0.20	194.74	492.00	1,243.03
7	30 seconds P = 175 mW with spray	3.35	0.19	923.49	2,224.79	5,359.79
8	30 seconds P = 175 mW dry	2.96	0.19	382.15	920.66	2,217.97
9	30 seconds P = 350 mW dry	3.25	0.19	738.35	1,778.77	4,285.26
10	60 seconds P = 175 mW with spray	3.50	0.19	1,322.84	3,186.87	7,677.54
11	60 seconds P = 175 mW dry	2.99	0.19	404.58	974.67	2,348.10
12	60 seconds P = 350 mW dry	2.33	0.19	89.68	216.04	520.47
13	120 seconds P = 175 mW with spray	3.06	0.19	471.17	1,135.10	2,734.59
14	120 seconds P = 175 mW dry	3.04	0.19	457.25	1,101.57	2,653.82
15	120 seconds P = 350 mW dry	2.44	0.20	107.99	272.82	689.28
16	240 seconds P = 175 mW with spray	2.90	0.19	331.17	797.82	1,922.05
17	240 seconds P = 175 mW dry	2.91	0.20	323.86	818.21	2,067.18
18	240 seconds P = 350 mW dry	2.13	0.19	56.63	136.44	328.69

* Mean log CFU: Mean logarithmic (base 10) value of colony-forming units (CFU).
† SE: Standard error of the mean log CFU.
‡ LB 95% CI: Lower boundary of the 95 percent confidence interval for the geometric (antilog) mean.
§ Geometric mean CFU: Antilog (base 10) of mean log CFU.
¶ UB 95% CI: Upper boundary of the 95 percent confidence interval for the geometric (antilog) mean.

and the high-wattage dry condition had log CFU values that were 0.67 log units lower than the low-wattage wet condition on average.

DISCUSSION

In this study, we evaluated the ability of an Er,Cr:YSGG laser with radially emitting laser tips to eliminate *E. faecalis* from dentinal tubules of prepared root sections. We chose *E. faecalis* as the test microorganism in this study owing to its high frequency of isolation from cases of failed endodontic treatment,[17,18] its resistance to calcium hydroxide treatment[20-22] and its relative insensitivity to laser irradiation.[28-30] We believed that if laser treatment was effective in eliminating this organism from infected dentinal tubules, we could

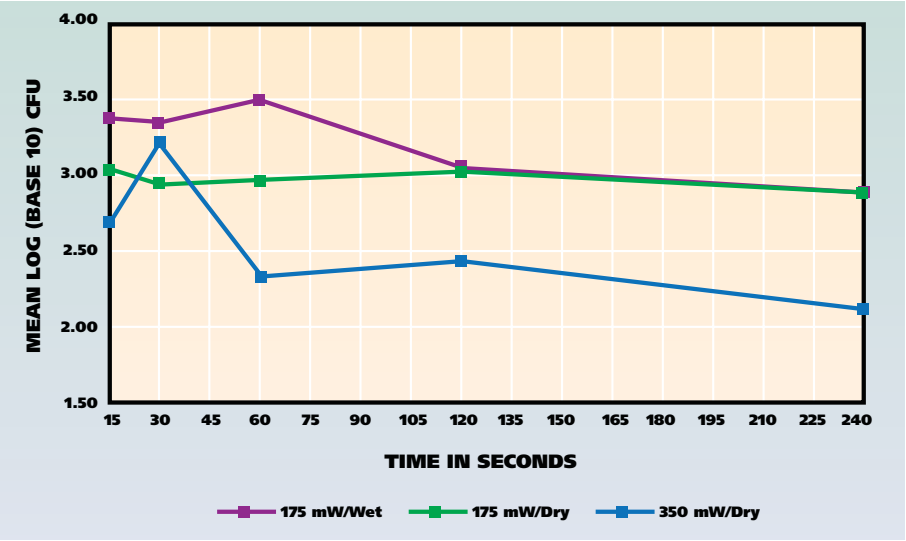

Figure 3. Plot of mean logarithmic scale colony-forming units (log CFUs) over time for the three laser treatment conditions. mW: Milliwatts.

TABLE 3

Mean colony-forming units: comparison with nontreated control group (group 1).

COMPARISON GROUP	MEAN DIFFERENCE (BASE 10 LOG SCALE)*	MEAN CFU AS % OF CONTROL MEAN†	SE‡	*P* VALUE§
2	–1.71	1.96	0.27	< .0001
3	–2.08	0.83	0.30	< .0001
4	–1.28	5.30	0.27	< .0001
5	–1.62	2.37	0.27	< .0001
6	–1.97	1.06	0.28	< .0001
7	–1.32	4.80	0.27	< .0001
8	–1.70	1.98	0.27	< .0001
9	–1.42	3.83	0.27	< .0001
10	–1.16	6.87	0.27	< .0001
11	–1.68	2.10	0.27	< .0001
12	–2.33	0.47	0.27	< .0001
13	–1.61	2.45	0.27	< .0001
14	–1.62	2.37	0.27	< .0001
15	–2.23	0.59	0.28	< .0001
16	–1.76	1.72	0.27	< .0001
17	–1.75	1.76	0.28	< .0001
18	–2.53	0.29	0.27	< .0001

* Mean difference: Difference in mean values of comparison and reference groups (base 10 logarithmic scale).
† Mean CFU as percentage of control mean: Antilog (base 10) of mean difference between experimental and control colony-forming units (CFU).
‡ SE: Standard error of the mean difference.
§ *P* value: *P* value of the mean difference.

infer that it would be effective against any organism found in endodontic infections, and that this technology might have clinical application in disinfecting root canals during endodontic therapy.

The penetration of the laser into the root dentin is governed by several factors. At the wavelength of the Er,Cr:YSGG laser (2.78 µm), there is absorption by dentin owing to the presence of hydroxide and interstitial water (dentin matrix and intratubular). On the basis of the fact that each laser pulse is composed of approximately 150 micropulses and each micropulse is responsible for the penetration of this energy of about 3 µm into water, depending on fluence, it is possible to achieve expansion of intratubular water and the collapse of water vapor as deep as 1,000 µm or more. This effect, known as "micropulse-induced sequential absorption," with expansion and collapse of water vapor, is capable of producing acoustic waves strong enough to disrupt intratubular bacteria. This penetration of dentin may provide the laser with advantages versus conventional methods of dentin disinfection, such as sodium hypochlorite irrigation, in cases in which limited access of the agent to the interstices of the root canal system may limit antimicrobial activity.

We evaluated the antimicrobial efficacy of the laser treatment by quantifying

posttreatment residual CFUs of *E. faecalis* from infected root dentin models on selective TA agar plates. On the basis of previous research[19] and our earlier pilot evaluations (R.H. Stevens, unpublished data, October 2004), we designed the models and experimental conditions in this study to allow for the infection, incubation and recovery of *E. faecalis* from infected dentinal tubules in an effective manner for evaluating the antimicrobial effect of laser and sodium hypochlorite treatment while minimizing the risk of false positive (uncounted bacteria) and false negative (contamination) results.

TABLE 4

Estimated regression coefficients and regression equations.*

PARAMETER/ CONDITION	REGRESSION COEFFICIENT	STANDARD ERROR	*P* VALUE	REGRESSION EQUATION
Intercept	3.59	0.191	< .0001	NA†
Time (Log CFU/Second)	–0.0021	0.00058	.0005	NA
Power (Log CFU/Watt)	–2.362	0.669	.0006	NA
From Dry to Wet (Log CFU)	0.254	0.116	.0297	NA
Wet Condition	NA	NA	NA	Log CFU = 3.59 – 0.0021 (time) – 2.36 (W)
Dry Condition	NA	NA	NA	Log CFU = 3.84 – 0.0021 (time) – 2.36 (W)

* The regression coefficient of –0.0021 for "time" shows that for each one-second increase in time, the logarithmic scale colony-forming units (log CFU) decreases by an average of 0.0021 base 10 log units. For the "watts" variable, the estimated log CFU value of –2.362 implies that as wattage increases from 0.175 to 0.350 W, the log CFU decreases by an average of 0.413 log units (2.362 × 0.175 = 0.413). The "wet" condition was coded as "0" and the "dry" condition as "1." Thus, for a change from dry (0) to wet (1), the log CFU increased by 0.254 log units on average. This is why the log CFU difference is 0.254 (3.84 – 3.59) between the wet and the dry equations, holding wattage and time constant.

† NA: Not applicable.

In a regression model presented in Table 4, we analyze and compare the effect of the three different laser treatment variables: time, wattage and wet/dry condition. According to the wet/dry equation, the dry method provided a more effective means of decreasing the CFUs, as indicated by the lower residual CFU number, which represents a larger percentage CFU reduction as compared with the control. Overall, the model showed that the largest reduction in CFUs occurred when time and wattage were at the maximum (240 seconds and 350 mW respectively) and when we used the laser in the absence of a water spray. This model is consistent with the results in Table 3, which shows that the greatest reduction in CFUs, when compared with the nontreated control (group 1), is found in laser group 18. Group 18 had the longest time of exposure (240 seconds in incremental steps) and the maximum wattage (350 ± 50 mW) and underwent the dry technique. This resulted in group 18's having the lowest percentage of residual CFUs, with only 0.29 percent of the CFUs of group 1. In other words, 99.71 percent of CFUs were eliminated (100 – 0.29 = 99.71 percent) by using the method of laser treatment.

Table 3 also shows that laser group 4—with only 15 seconds of laser exposure, wattage of 175 ± 25 mW and air-water spray—had 5.3 percent of the CFUs of the control group 1. The 94.7 percent (100 percent – 5.3 percent = 94.7 percent) still is considered a significant reduction in *E. faecalis* bacteria. However, the method used for group 18 was 18.27 times (0.29 mean CFU as a percentage of the control mean) more efficient than the group 4 method (5.3 mean CFU as a percentage of the control mean) in reducing the number of CFUs.

For each time point (except the 30-second treatment), the 350 ± 50 mW laser dry treatment group consistently showed the lowest number of residual CFUs recovered and lowest percentage as compared with the control group. These results were statistically significant. The effective results obtained for the 350 mW dry laser treatment may be attributed to the direct effect of the laser on water-containing bacteria. When water spray is used, the laser energy applied to the root canal dentin is diminished owing to this laser wavelength's high absorption properties. This effect may explain the decreased effectiveness in bacterial reduction among the laser groups that used water spray. Some treatment groups—such as laser groups 6, 8 and 9—showed low CFUs at low treatment times of 15 and 30 seconds of exposure (Table 4). It is possible that even at these shorter times, the addition of laser power was capable of eliminating most of the cultivable *E. faecalis* in the models.

The reason for the inconsistent result for the 30-second laser treatment group (group 9) is not clear. The two sodium hypochlorite–treated groups difference from each other by more than twice (2.36 times) the mean CFU percentage of the control. The results were statistically significant when compared with the control but not significant when compared with each other.

The base 10 log scale of all three lased groups compiled together over time indicated a tendency for lower CFUs and higher percentage reductions of bacteria at higher treatment times, with some variability in data mentioned previously (at 15 seconds and 60 seconds). We found the overall percentage bacterial reduction in all laser treatment groups to be statistically significant as compared with the control for all groups and to other treatment groups in same cases.

The fiber tips used in this study were designed specifically to provide effective radial laser emission. The geometry was conical, with a cone angle of 60 ± 5 degrees. The energy density delivered at the root canal surface for the two output powers used was 8.12 joules per square centimeter for the 175 mW setting and 17.4 J/cm^2 for the 350 mW setting. Heat generation from this energy density could be a problem in terms of deleterious effects on the root surface. We conducted separate tests to measure the temperature increase in the absence of water cooling at the highest power setting. We recorded the temperature continuously with the laser firing for five seconds on and approximately 10 seconds off. The maximum temperature rise under these conditions, measured at approximately 500 µm from the canal wall, was 2.6 C during continuous recording (data not shown). For thicker sections of the root, the temperature rise at the root surface would be even lower. According to Eriksson and Albrektsson,[31] an increase of temperature that is less than 10 C is considered safe for osseous tissue.

Jha and colleagues[32] conducted a study examining the antimicrobial effects of the Er,Cr:YSGG laser. In that study, the investigators concluded that the "Er,Cr:YSGG laser instrumentation was [not] able to eliminate an *E. faecalis* infection in root canals" and that "the laser was completely ineffective in disinfecting root canals when sterile saline was used as an irrigation solution." While our results are in agreement with the first statement, they are in sharp contrast to the second. In contrast to the laser's being "completely ineffective in disinfecting root canals," we found that a high degree of disinfection (99.7 percent) could be achieved by using the Er,Cr:YSGG laser. The difference in the results may be attributed to differences in the methodology used in the two studies. In the Jha and colleagues[32] study, the researchers recovered residual viable bacteria after laser treatment of infected root dentin by collecting dentin shavings from the root canal wall. They then transferred dentin shavings to broth tubes and incubated them. The development of turbidity was taken as evidence of bacteria survival of the lasing treatment. However, this model is not quantitative in the sense that a single surviving bacterial cell from the infected dentin would give precisely the same result as would a million surviving organisms: in both cases, the tube receiving the infected dentin shavings turned turbid following incubation. Therefore, according to this model, it would be impossible to determine whether any reduction (disinfection) of the bacterial population had occurred. In our model, the surviving bacteria were quantified by immediately diluting 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. Consequently, although we did not find total elimination of viable organisms (that is, sterilization), we did achieve a significant reduction in the viable bacterial load, approaching sterility.

Although we did not find total elimination of viable organisms, we did achieve a significant reduction in the viable bacterial load, approaching sterility.

Our results suggest that the Er,Cr: YSGG laser may be a valuable tool for root canal disinfection of *E. faecalis* when one uses radially emitting laser tips. One benefit of the laser over conventional treatment is that it has the ability to achieve significant disinfection of canals infected with *E. faecalis*, for which there is evidence that conventional calcium hydroxide is not as effective, owing to the resistance of this type of bacterium. Should modification of the lasing procedure permit predictable, total elimination of viable bacteria in the dentin, this could justify a one-visit endodontic treatment for infected root canals. Other potential benefits of using the laser include

conservation of root structure and less emphasis on mechanical instrumentation, especially in curved roots. This benefit is promising, as the laser tips are flexible and come in sizes as small as 200 µm in diameter (equal to the diameter of the tip of a no. 20 file), both of which would minimize length of procedure and dependence on mechanical instrumentation. These tips are capable of penetrating narrow, long and curved canals more efficiently, in areas that sodium hypochlorite irrigation may not be able to reach. The results of our study show that the Er,Cr:YSGG laser is able to disinfect dentinal tubules of straight roots enlarged to the diameter of a no. 4 Peeso-type reamer using a 200-µm radially emitting laser tip for 15, 30, 60, 120 and 240 seconds of cumulative exposure. More studies are needed to test the ability of the laser to disinfect curved roots that have been instrumented minimally before dentinal tubule infection in our model system.

One of our goals was to determine if either the chemical disinfection or the laser treatments under specified conditions are capable of a 100 percent reduction in infection. None of the treatment conditions was able to demonstrate such effects. The dry technique at 240 seconds of cumulative laser exposure came the closest to this objective, with a mean residual CFU percentage of 0.29 percent, which was 2.86 times lower than the most effective sodium hypochlorite (3-mL) disinfection. Further studies to evaluate new treatment protocols that could count for completed bacterial eradication need to be considered in the future.

From all that we know of pulpal and periapical disease, the elimination of infection (that is, sterilization) and prevention of subsequent infection is at the heart of endodontic therapy. To date, no existing procedure allows the clinician to sterilize an infected root canal system quickly and easily and with absolute surety. Therefore, the goal of our work was to learn whether the use of this particular laser system (Er,Cr:YSGG) could reliably accomplish this goal of root canal sterilization. While our results did not demonstrate complete elimination of infection from our root dentin models, our test system did allow us to quantify the degree of change in bacterial load after laser treatment. We found that we could achieve a 99.7 percent (nearly 3-log) reduction in viable bacteria using the laser. Given the bacterial load typically reported to be present in infected root canal systems (10^3-10^5 CFU), a 3-log decrease in titer is a significant reduction in that it approaches the goal of complete elimination of infection. Clearly, more work needs to be done; however, our results are encouraging and suggest that we are at least close to our goal.

CONCLUSIONS

We found statistically significant differences between all groups as compared with the control. The treatment with the lowest percentage mean CFU as compared with the control—0.29 percent—was the dry treatment lasting 240 seconds with the laser at $P = 350 \pm 50$ mW. The next groups to follow were group 12 (60 seconds, $P = 350 \pm 50$ mW, dry) with 0.47 percent and group 15 (120 seconds, $P = 350 \pm 50$ mW, dry) with 0.59 percent. The sodium hypochlorite, 3-mL group ranked as the fourth best with 0.83 percent. Overall, the laser showed better results than the sodium hypochlorite did, but none of the treatments demonstrated complete elimination.

The Er;Cr: YSGG laser employing radially emitting laser tips demonstrated a considerable effect on bacterial reduction within dentinal tubules of roots infected with *E. faecalis*. The effect depended on the time, wattage and technique (wet versus dry), each variable being used as a separate predictor. Our study demonstrated that laser treatment with radially emitting tips could be considered as an alternative method for root canal disinfection of *E. faecalis* in endodontic treatments. ■

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ORIGINAL ARTICLE

The impact of an erbium, chromium: yttrium-scandium-gallium-garnet laser with radial-firing tips on endodontic treatment

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Abstract Radial-firing tips should allow a more homogeneous laser irradiation of root canal walls. The aim of the study was to assess the effects of erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser irradiation in conjunction with those newly designed tips. The investigation comprised bacteriology, morphological evaluations and temperature measurements. Root canals were inoculated with two test strains and laser irradiated with power settings of 0.6 W and 0.9 W and a repetition rate of 20 Hz. Subsequently, the samples were subjected to microbiological evaluation. The morphological changes of the canal walls were assessed by scanning electron microscopy. To reveal possible thermal side effects, we carried out temperature measurements. The bacteriological evaluation revealed a decisive disinfectant effect. Scanning electron microscopy showed the homogeneous removal of smear layer from the root canal walls. The temperature rise at the root surface during the irradiation was moderate, yielding 1.3°C for the 0.6 W setting and 1.6°C for the 0.9 W setting. The investigations indicated that the Er,Cr: YSGG laser, in conjunction with radial-firing tips, is a suitable tool for the elimination of bacteria in root canals and for the removal of smear layer.

Keywords Endodontics · Root canal · Laser · Radial-firing tips · Bacteriology · Scanning electron microscopy

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Introduction

Since bacteria are the most important elicitors of periapical infections, the decisive objective in endodontic therapy is the disinfection of the root canal and the three-dimensional network of dentinal tubules. From the infected pulp tissue bacteria penetrate into the deeper layers of root dentine and propagate a periapical inflammation with subsequent destruction of the adjacent connective tissues [1–3].

The local microenvironment favours the selection of relatively few bacterial species, which can survive and proliferate, being out of reach of the host's immune response [4–8]. Even rinsing solutions applied during conventional root canal treatment only partly affect those bacteria. The pathogenic microorganisms are able to penetrate the root dentine up to a depth of more than 1 mm, whereas disinfecting solutions reach a depth of only approximately 100 μm [9, 10]. In addition, bacteria such as *Enterococcus faecalis* have the capability to form intra- and extra-radicular biofilms, which makes them even harder to control [11–13]. These facts are often responsible for those cases that are therapy resistant from the beginning or that

end up as long-term failures after endodontic treatment has been accomplished.

Considering this, the disinfection of the root canal, including the most distant areas of the tubular system, can be regarded as a major challenge in today's endodontic treatment and is of fundamental importance for the prolonged preservation of endodontically treated teeth. The use of lasers in the field of endodontology represents an innovative approach to match these requirements. In general, dental lasers provide greater accessibility of formerly unreachable parts of the tubular network, due to their better penetration into dentinal tissues [14–16]. Although a wide spectrum of wavelengths has been investigated since the early 1980s, the neodymium: yttrium-aluminium-garnet (Nd:YAG) laser can be regarded as the best-established system in endodontic treatment. Owing to the laser's wavelength of 1,064 nm, flexible conductors can be used for application in narrow and bent root canals. This laser yields a bactericidal effect not only on root canal surfaces but also in the deeper layers of dentine. Several studies by White et al. [17], Rooney et al. [18], Gutknecht et al. [19], Moritz et al. [20] and Schoop et al. [21] have proved the high bactericidal effect of the Nd:YAG laser.

Diode lasers are comparable to the Nd:YAG laser in terms of effectiveness. They emit at a wavelength of 810 nm or 980 nm and possess satisfying bactericidal capabilities, as shown by Moritz et al. [22] and Schoop et al. [21, 23].

For the removal of dental hard tissue the erbium:yttrium-aluminium-garnet (Er:YAG) and the erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) lasers provide suitable wavelengths. Emitting at 2,940 nm and 2,780 nm, respectively, these lasers act through photoablation, since their wavelengths correlate closely with the absorption maximum of the water contained by the hydroxyapatite. When irradiated, water contained in the dental hard tissue evaporates instantaneously and thereby ablates the surrounding tissue with only minimal thermal side effects. This has been demonstrated in a study by Hibst and Keller [24].

Although primarily used for the preparation of dental hard substances, the erbium wavelengths can also be applied in the field of endodontic treatment. The development of flexible fibre tips allows the irradiation of even narrow or bent root canals. Hibst et al. [25] proposed the use of the Er:YAG laser in endodontics, and later studies by Schoop et al. [21, 26, 27] confirmed the laser's qualification.

Several papers have focused on caries removal and cavity preparation using the Er,Cr:YSGG laser [28–30], whereas authors such as Yamazaki et al. [31] and Kimura et al. [32] described the morphological changes encountered in irradiated root canal walls. A study by Schoop et al. [21] illustrated the bactericidal potential of the Er,Cr:YSGG laser applied to root dentine samples.

Although favourable results have been achieved with all those wavelengths, the delivering fibre tips still show some room for improvement. Owing to total reflectance at the fibre walls, the laser beam is expanded to a certain degree when leaving the end of the fibre tip. However, the biggest part of the laser light will still be propagated straight towards the apex of the root. By conducting the irradiation of the root canal in spiral movements and through a certain tilting of the fibres, one can minimize this effect, to a certain extent, and a sufficient energy density at the root canal walls can be achieved. Striving for the improvement of the established delivery systems, a new generation of fibre tips has been developed that allow a more homogeneous irradiation of the root canal walls. The ends of these radial-emitting fibre tips show a conical outline with a cone angle of 60°. The laser light, therefore, is expanded to a broad cone, facilitating an even coverage of the whole root canal wall.

This study examined the bactericidal, morphological and thermal effects of the Er,Cr:YSGG laser, utilizing these radial-firing tips in root canals. To evaluate the antimicrobial effect of the laser, we performed bacteriological in vitro experiments with two different species. Morphological alterations on dentinal surfaces were recorded by use of an environmental scanning electron microscope (ESEM), and the thermal effects caused by laser irradiation were measured with a thermocouple.

Materials and methods

Sample preparation

Sixty extracted human teeth with one root were endodontically prepared. The teeth were stored in physiological saline solution after the extraction. Subsequently, trepanation and orthograde enlargement of the root canal to ISO 70 were performed. During the preparation process, the root canals were rinsed with physiological saline solution only; no EDTA was applied. The prepared teeth were assigned to six different experimental groups and treated accordingly.

Bacterial inoculation

The samples were steam sterilized (Melatronic 23, Melag, Berlin, Germany) at 134°C for 10 min for the removal of all pre-existing germs. Following this step, the root canals were inoculated with 10 μl of either of the two test strains, *Escherichia coli* (ATCC 10536) or *Enterococcus faecalis* (ATCC 29212) with a micropipette. The initial bacterial count was 10^8 colony forming units per millilitre (CFU/ml).

The inoculated teeth were sealed with wax and then placed into sterile microcentrifuge tubes containing 100 μl of a physiological saline solution. After an incubation period of 4 h at 35°C, the teeth were taken out of the Eppendorf tubes, and the wax seal was removed.

Laser irradiation

Laser irradiation was performed in the root canal. An Er,Cr: YSGG laser (Waterlase MD, Biolase, San Clemente, USA), emitting at a wavelength of 2,780 nm, was used. In this device, pulse energy can be varied between 25 mJ and 300 mJ, and the repetition rate can be adjusted between 10 Hz and 50 Hz. This results in an output power of 0.5–8 W. For our investigation, the device was equipped with exchangeable 200 μm fibre tips with a conical outline, allowing radial emission of the laser beam.

The actual laser power emitted at the fibre tip was measured by a wattmeter (FieldMaster, Coherent Inc., Auburn, CA, USA) before each irradiation to ensure stable and standardized power outputs. Laser irradiation was performed in the root canal. For each strain, 20 samples were treated. Two groups of ten samples each underwent laser treatment at a setting of 2 W (100 mJ) and 3 W (150 mJ) as indicated on the display of the laser unit, corresponding to an actual power output of 0.6 W (30 mJ) and 0.9 W (45 mJ), measured directly at the end of the fibre tip. The pulse rate was the same for all groups (20 Hz). The rather big difference between the laser setting and the actual power output can be explained by the calibration factor of the fibre, which ranges around 70%. That means that roughly two-thirds of the laser energy are absorbed within the fibre tip.

Each sample was treated with one lasing cycle, which comprised five irradiations of 5 s duration with a 20 s break in between. For irradiation the optical tip was inserted as far as the apex. Then, the laser was activated, and the root canal was continuously radiated from apical to coronal, in slow, circling movements. By means of this procedure the irradiation of the entire root canal could be ensured. For each test microorgansim, ten samples served as a control group. Those samples were treated the same way as the actual laser samples, except for the very irradiation itself. That is to say, the laser fibre was introduced into the canal without activating the laser device.

The irradiation was done by hand and always by the same investigator to ensure comparability between the sample groups within the actual study and preceding investigations.

Bacteriological evaluation

Immediately after the laser treatment the root canal was rinsed with 1 ml of a physiological saline solution, and the eluate was collected in a microcentrifuge tube. Finally, the bacterial count was determined. The extracted fluid was diluted in log 10 steps. From each dilution, 20 μl was applied to culture plates (sheep agar plates, bioMérieux, Marcy l'Etoile, France) and incubated for 24 h at 37°C. The colonies were then counted, and the total number of bacteria (colony forming units per millilitre of the extraction fluid) was assessed. The lowest detection level of bacteria was 5×10^2 CFU/ml.

Temperature measurements

To assess the thermal impact of the Er,Cr:YSGG laser irradiation, we measured the temperatures. For this purpose, five samples were used for each power setting. The teeth were mounted on an even thermocouple (manufactured by the Technical University of Vienna and provided with a digital thermometer) measuring 10 mm by 10 mm using a silicon-based heat-conductive compound (Dow Corning 340 Heat Sink Compound, Dow Corning, Midland, Michigan, USA). During the irradiation procedure, which was carried out in the same way as the irradiation of the inoculated samples, the maximum temperature increase (starting from a room temperature of 21°C) was recorded by a digital thermometer (TMG-1 device, manufactured by the Technical University of Vienna) with a sampling rate of 20 Hz and a sensitivity of 0.1°C. The average value and the standard deviation of the five measurements per laser setting were calculated subsequently.

Environmental scanning electron microscopy

An additional 20 samples were subdivided into two groups (0.6 W and 0.9 W each) and prepared as described above (except for the bacteriological procedure). The samples were cut longitudinally with a diamond-coated band saw ("Trennschleif System", Exakt, Norderstedt, Germany) then submitted to scanning electron microscopy so that we could evaluate the morphological changes induced by the laser irradiation. The specimens were assessed with an environmental scanning electron microscope (ESEM XL30, Philips, Eindhoven, The Netherlands) working with mild negative pressure and without sputtering of the samples, thus facilitating the assessment of native samples and the minimization of artefacts. Pictures were taken at different magnifications.

Results

Bacteriology

Table 1 shows the results of the bacteriologic tests on *Escherichia coli* and *Enterococcus faecalis*.

Table 1 Bacterial counts of *Escherichia coli* and *Enterococcus faecalis*. For each irradiation power applied, the number of specimens and the range of CFUs/ml is indicated

Parameter	*Escherichia coli* (CFU/ml)						*Enterococcus faecalis* (CFU/m)l					
	Below detection level	10^3	10^4	10^5	10^6	10^7	Below detection level	10^3	10^4	10^5	10^6	10^7
Control				6	4					3	6	1
Er,Cr:YSGG; 0.6 W	5	5					5	2	1	2		
Er,Cr:YSGG; 0.9 W	10						4	4	2			

Samples are rated in log-steps of the colony counts (CFU/ml), and the specific radiation power applied.

The results of the control group of both test strains showed colony counts ranging between 10^5 CFU/ml and 10^6 CFU/ml, demonstrating a decrease of 2 to 3 log-steps through the inoculation and incubation process and the further processing of the samples.

As far as *Escherichia coli* was concerned, the Er,Cr:YSGG laser succeeded in a major reduction of the test bacterium, even at the lower output power of 0.6 W. At the higher power value (0.9 W), the impact was even more considerable, yielding a complete reduction to below the detection level.

The laser device tested was also effective in reducing the gram positive *Enterococcus faecalis*. At 0.6 W the Er,Cr:YSGG laser was capable of removing the germ to an extent of 3 to 4 log-steps (compared with the control group) in a major part of the samples. The higher output power (0.9 W) conferred only a slight improvement in terms of disinfectant effectiveness compared with the sample group irradiated with a power of 0.6 W. For *Enterococcus faecalis*, complete reduction to below the detection level was, however, achieved in none of the groups.

Temperature measurements

When an output power of 0.6 W was chosen, the irradiation of the samples resulted in an average temperature rise of 1.3°C at the root surface. The higher output of 0.9 W yielded an average temperature rise of 1.6°C at the root surface.

Table 2 Temperature rise at the root surface. The averages and standard deviations have been calculated from five individual measurements per power setting

Device	0.6 Watt	0.9 Watt
Er,Cr:YSGG	1.3°C±0.4°C	1.6°C±0.5°C

Table 2 presents the temperature measurements. All the measurements were carried out at a room temperature of 21°C; thus, they refer to an initial sample temperature of 21°C. For instance, the value 1.3°C stands for a temperature rise to 22.3°C. Although a higher irradiation power results in a stronger temperature increase at the sample surface, the temperature stays within safe boarders.

Environmental scanning electron microscopy

Figure 1 shows a length cut through a root that has been irradiated with the Er,Cr:YSGG laser at 0.6 W using a radial-firing tip (magnification ×100). At the left and right margins of the picture, the cut surface of the root dentine can be seen. The root canal surface exhibits the typical rough structure after the removal of the adhering smear layer.

Figure 2 gives a detailed view of the root canal wall at 2,000-fold magnification after irradiation with 0.6 W. The dentinal tubules have been partly exposed; other portions of the root canal wall are still covered with a thin smear layer.

When the output power was increased to 0.9 W, the low magnification reveals the same basic surface structure as depicted in Fig. 1. The cut surfaces of the root dentine are discernible alongside a comparably clean and even surface (Figure 3).

Figure 4 shows a detail of a root canal irradiated with 0.9 W. Most of the dentinal tubules have been exposed, and no cracks or signs of melting can be discerned.

Discussion

Successful endodontology relies, to a great extent, on complete cleaning of the root canal. Infected dentine and pulpal tissue can endanger therapy outcome. Conventional root canal treatment aims at the removal of the infected pulp and dentine layers by using mechanical techniques and bactericidal irrigants. In this context, the method of

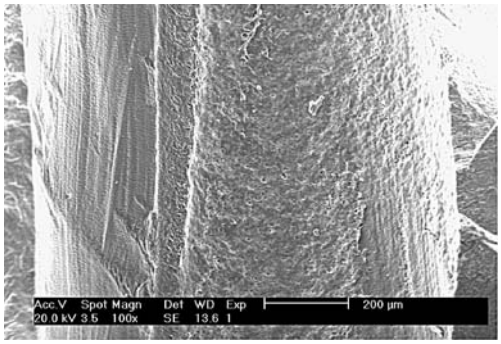

Fig. 1 SEM picture of a root canal irradiated with an output power of 0.6 W. Magnification ×100

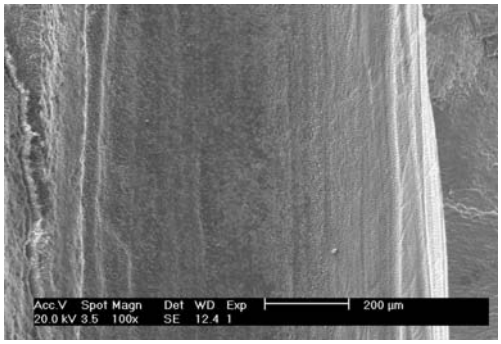

Fig. 3 SEM picture of a root canal irradiated with an output power of 0.9 W. Magnification ×100

bactericidal rinsing encounters a major problem: studies by Kouchi et al. [9] have shown that bacteria colonize the periluminal dentine up to a depth of 1,100 µm. Chemical disinfectants penetrate only 100 µm into the dentine, as indicated by Berutti et al. [10]. In addition, bent root canals or side branches can be obstacles in the conventional root canal treatment. The utilization of lasers helps to overcome this issue, as already pointed out in the Introduction section. The high penetration depth of the laser light in the dentinal tissue seems to be the most appropriate explanation for the satisfying bactericidal effect of different laser wavelengths. One possible explanation for this kind of light propagation is given by Vaarkamp et al. [15], Odor et al. [16] and Kienle et al. [33]. These authors have described the ability of enamel prisms and dentine tubules to scatter light within dental hard tissues. In fact, it was possible to demonstrate the effect of Nd:YAG laser irradiation on bacteria through indirect irradiation [34].

The use of laser wavelengths suitable for the preparation of dental hard substances could add another interesting aspect to the field of root canal cleaning. In an in vitro study [27], Schoop et al. described the impacts of Er:YAG laser irradiation on root canal walls and the bactericidal effect of the wavelength. A comparative study confirmed the high bactericidal potential of both the Er:YAG- and the Er,Cr:YSGG lasers [21].

Irrespective of the wavelength utilized, the beam geometry at the fibre output corresponds to a narrow cone, delivering the highest radiation density straight towards the apex. Considering the fact that the diameter of the instrumented root canal is rather larger than the fibre diameter, a larger portion of the laser beam can be directed at the root canal walls just by tilting the fibre tip during the irradiation procedure. Although a satisfying bactericidal effect can thus be achieved, the light distribution on the canal surface still seems to be rather irregular. In fact, SEM investigations of root canal surfaces that have been irradiated with Er:YAG- or Er,Cr:YSGG lasers show isolated irregularities like molten and recrystallized portions of dentine or a distinct crack formation [21, 26, 27].

Through the introduction of a new radial-firing fibre tip, the mode of light emission in the root canal has been improved. Owing to the conical shape of the fibre tip, the laser light is emitted in the form of a broad cone with an angle of about 60°, according to the manufacturer's information, allowing a more uniform coverage of the whole dentinal surface.

The aim of our study was to evaluate the effectiveness of Er,Cr:YSGG laser irradiation applied through a radial-firing fibre tip with a diameter of 200 µm. Owing to the comparably high attenuation of the laser beam by the fibre tips, the effective output evaluated was 0.6 W and 0.9 W,

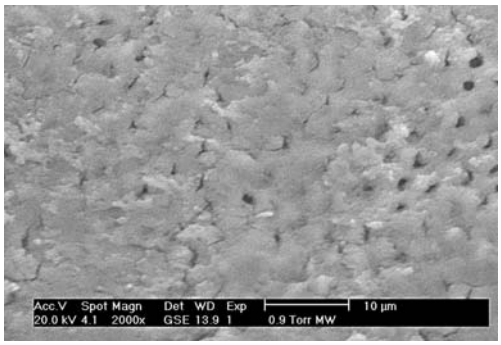

Fig. 2 SEM picture of a root canal irradiated with an output power of 0.6 W. Magnification ×2,000

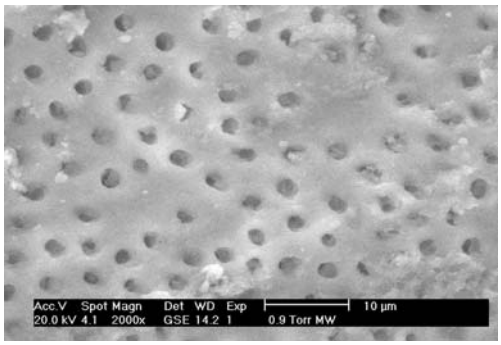

Fig. 4 SEM picture of a root canal irradiated with an output power of 0.9 W. Magnification ×2,000

as explained above in Materials and methods, Laser irradiation.

In fact, the higher setting had to be applied in order to achieve a reduction of *Escherichia. coli* to below the detection level in all samples. When the samples with *Enterococcus faecalis* were irradiated, again a remarkable antibacterial effect was observed; complete reduction to below the detection level was, however, not accomplished in all samples. The results achieved are comparable to those described in an earlier study [35], where conventional 300 μm fibre tips were applied in conjunction with a remarkably higher power output (1 W and 1.5 W, respectively).

On the other hand, the negligible temperature rise at the root surface illustrates the low energy dosage delivered to the samples. The temperature rise at the root surface did not exceed 1.6°C; therefore, possible damage to the surrounding periodontal tissues could be excluded. In addition, excessive heating of the entire sample obviously could not be regarded as the decisive reason for the bactericidal effect.

Owing to the high absorption of the Er,Cr:YSGG laser's radiation in water, the penetration depth in dentine should be rather restricted, in contrast to other lasers such as the Nd:YAG or the diode. One possible explanation for the good bactericidal effect in this study could, therefore, be a lack of penetration of the test bacteria into the dentinal tubules, because the samples were incubated only for 4 h prior to laser irradiation. However, the Er:YAG- and the Er,Cr:YSGG lasers have also shown good bactericidal potential when the irradiation has been carried out indirectly through a dentine layer of 1 mm [21]. Another explanation for the effect, particularly on *Enterococcus faecalis*, could be a certain degree of conduction of the laser light within the dentinal tubules, resulting in a higher penetration depth. Other factors, such as shock waves or cavitation effects, have at least been reported for other laser devices [36, 37] and could represent a further explanation for the actual impacts of Er,Cr:YSGG laser irradiation. In any case, further investigations are necessary to clarify the exact interactions of laser light and bacteria.

Scanning electron microscopy revealed the ability of the Er,Cr:YSGG laser applied with a radial-firing tip to remove smear layer and debris from the root canal wall and to open up the orifices of dentinal tubules. This effect should facilitate tight root canal sealing. We used the laser as an adjunct to conventional root canal preparation. Although the laser was applied without water spray, a very homogeneous impact on the root canal walls, with no signs of melting or cracking, was observed. This leads us to the conclusion that the expansion of the beam by the tip geometry favours a higher energy distribution at the root canal walls.

Although the laser was able to reduce strongly the number of viable bacteria in the root canal, the bactericidal potential was slightly inferior compared to the same device applied through a conventional 300 μm fibre tip. Since the total temperature rise was negligible, there seems to be room for a laser application through radial-firing tips with a higher diameter of 300 μm or 400 μm, allowing a higher energy output ranging around 1.5 W. When those fibre tips become available, additional investigations will be necessary.

Conclusion and clinical relevance

Considering all the facts described, one can conclude that the wavelength and delivery system tested in our study may be suitable for the cleaning and disinfection of root canals and can be safely applied, if the common precautions for laser application are observed and the applied energy and irradiation time stay within the proposed range. For the results to be confirmed further, and for the wavelengths to be investigated under in vivo conditions, clinical studies are necessary.

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The use of the erbium, chromium:yttrium-scandium-gallium-garnet laser in endodontic treatment

The results of an in vitro study

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One objective in endodontic therapy is to sanitize the root canal and the three-dimensional network of dentinal tubules. Bacteria from infected pulp tissue can penetrate into the deeper layers of root dentin and propagate periapical inflammation with subsequent destruction of the adjacent connective tissues.[1-3]

The local microenvironment of the root canal system favors the selection of a few bacterial species that can survive and proliferate when they are out of reach of the host's immune response.[4-8] Rinsing solutions used during conventional root canal treatment affect those bacteria only partially. The pathogenic microorganisms are able to penetrate the root dentin up to more than 1 millimeter, whereas rinsing solutions reach a depth of around only 100 micrometers.[9,10] In addition, bacteria such as *Enterococcus faecalis* are able to form intra- and extraradicular biofilms, which makes it even harder to con-

ABSTRACT

Background. The use of the erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser has become accepted in the field of cavity preparation. The development of miniaturized and flexible fiber tips has allowed this device to be used in endodontics. The authors conducted an in vitro study to assess the effects of Er,Cr:YSGG laser irradiation on root canals.

Methods. The authors inoculated root canals with two bacteria, laser irradiated them at two power settings and subjected them to a quantitative microbiological evaluation. They used scanning electron microscopy (SEM) to assess morphological changes in endodontically processed and laser-irradiated root canal walls. They measured temperature increases on the root surface to determine possible thermal side effects.

Results. The bacteriological evaluation revealed a disinfecting effect in the root dentin samples that was dependent on the output power but not specific for the bacterial species investigated. SEM showed the removal of the smear layer from the root canal walls and the exposure of dentinal tubules. The temperature rise during irradiation was moderate when standardized power settings were used.

Conclusions. The Er,Cr:YSGG laser can be used to eliminate bacteria in root canals. It also effectively removes smear layer and debris from the canal wall.

Clinical Implications. Practitioners can use the Er,Cr:YSGG laser to prepare root canals for endodontic therapy.

Key Words. Endodontics; root canal; laser; bacteriology; scanning electron microscopy.

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trol them.[11-13] These facts often are the reasons for cases that are resistant to therapy from the beginning or end up as long-term failures after endodontic treatment.

Considering this, disinfecting the root canal—including the most distant areas of the tubular system—is a major challenge in endodontic treatment and is of fundamental importance for the prolonged preservation of endodontically treated teeth. The use of lasers in the field of endodontics is an innovative approach for meeting these requirements. In general, dental lasers provide access to formerly unreachable parts of the tubular network, owing to the fact that they penetrate dental tissues better than rinsing solutions.[14-16]

Since the early 1980s, several studies on the impact of different laser systems on the root canal and the surrounding dentin have been published. The carbon dioxide (CO_2) laser, which emits a wavelength of 10,600 nanometers, has been used in surgery for a long period. In 1986, Zakariasen and colleagues[17] showed for the first time that this wavelength could be used in endodontics with a good bactericidal effect. In 1995, Moritz and colleagues[18] achieved a partial closure of dentinal tubules using the CO_2 laser on root canal surfaces. Owing to the fact that the emitted long wave infrared radiation (10,600 nm) can be transmitted into the root canal exclusively by using a rigid hollow wave guide, the canal lumen must be well-prepared and the laser can be used only in straight root canals.

An in vitro study by Pini and colleagues[19] focused on the use of the xenon chloride (XeCl) excimer laser, which emits ultraviolet radiation at 308 nm. This low wavelength leads to a satisfactory removal of hard tissues and a bactericidal effect with only limited thermal side effects. The requirements of technical resources are tremendous and, therefore, the use of the XeCl excimer laser remains restricted primarily to basic research.

Moshonov and colleagues[20] demonstrated the efficacy of the argon laser in removing intracanal debris by means of computerized scanning electron microscopy (SEM), whereas Blankenau and colleagues[21] illustrated this procedure's safety regarding the temperature rise at the root surface when they used an argon laser at power settings of 1 and 2 watts.

The most widely used laser in endodontics is the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser, which emits a wavelength of 1,064 nm. Owing to the wavelength's being in the near infrared range, flexible conductors can be used in narrow and curved root canals. This laser yields a bactericidal effect on root canal surfaces and in the deeper dentin layers. Studies by White and colleagues,[22] Rooney and colleagues,[23] Gutknecht and colleagues[24] and Moritz and colleagues[25] showed the high bactericidal effect of the Nd:YAG laser.

The diode laser is comparable to the Nd:YAG laser in terms of effectiveness. It emits at a wavelength of 810 nm and has comparable bactericidal capabilities.[26]

For the removal of dental hard tissue, the erbium:yttrium-aluminum-garnet (Er:YAG) and the erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) lasers provide suitable wavelengths. Emitting at 2,940 nm and 2,780 nm, respectively, these lasers act through photoablation since their wavelengths correlate closely with the absorption maximum of hydroxyapatite. When irradiated, water contained in the dental hard tissue evaporates instantaneously and ablates the surrounding tissue with only minimal thermal side effects.[27]

Although used primarily for the preparation of dental hard substances, the erbium lasers also can be used in endodontic treatment. The development of superior light-conductive materials allows for the irradiation of narrow or curved root canals. Hibst and colleagues[28] proposed that Er:YAG lasers be used in endodontics; later studies by Schoop and colleagues[29,30] confirmed Er:YAG laser's qualification.

Some articles focused on caries removal and cavity preparation using the Er,Cr:YSGG laser,[31-33] while Yamazaki and colleagues[34] and Kimura and colleagues[35] described the morphological changes encountered in irradiated root canal walls.

In our in vitro study, we examined the bactericidal, morphological and thermal effects of the Er,Cr:YSGG laser when it is used in addition to root canal treatment. To evaluate the antimicrobial effect of the laser, we performed bacteriolog-

ABBREVIATION KEY. CO_2: Carbon dioxide. **Er,Cr:YSGG:** Erbium, chromium:yttrium-scandium-gallium-garnet. **Er:YAG:** Erbium:yttrium-aluminum-garnet. **ESEM:** Environmental scanning electron microscope. **Nd:YAG:** Neodymium:yttrium-aluminum-garnet. **SEM:** Scanning electron microscopy. **XeCl:** Xenon chloride.

ical experiments in vitro with two different bacterial species. We used an environmental scanning electron microscope (ESEM) to record morphological alterations on dentinal surfaces, and we used a thermocouple to measure the thermal effects caused by laser irradiation.

MATERIALS AND METHODS

Sample preparation. We stored 60 extracted human teeth with one root each in physiological saline solution (that is, saline of the same concentration as that in the human body). We then performed trepanation and orthograde enlargement of the root canal to International Organization for Standardization standard 70. We assigned the prepared teeth to six experimental groups.

Bacterial inoculation. We steam-sterilized the samples at 134 C for 10 minutes to remove all pre-existing bacteria. We then inoculated the root canals with 10 microliters of either of the two test bacteria—*Escherichia coli* (American Type Culture Collection 10536) or *E. faecalis* (American Type Culture Collection 29212)—by means of a micropipette. The initial bacterial count was 10^8 colony-forming units per milliliter (CFU/mL). We sealed the inoculated teeth with wax and placed them into sterile microcentifuge tubes containing 100 µL of physiological saline solution. After incubating the teeth for four hours at 35 C, we took them out of the microcentifuge tubes and removed the wax seals.

Laser irradiation. One investigator (U.S.) performed all of the irradiations to ensure comparability between the test samples in our study and those of preceding investigations.[29,30] He performed laser irradiation in the root canals by using an Er,Cr:YSGG laser that emitted a wavelength of 2,780 nm. The pulse energy of the Er,Cr:YSGG laser varied between 25 and 300 millijoules at a fixed repetition rate of 20 hertz, which resulted in an output power of 0.5 to 6 W. The laser was equipped with exchangeable fiber tips that had a diameter of 300 µm. The investigator measured the laser power emitted at the fiber tip by using a wattmeter before each irradiation to ensure stable and standardized power outputs.

For each test bacteria, he used the following experimental protocol. He treated one group of 10 samples with the laser set at 180 mJ as indicated at the display of the laser unit, with an output power of 1 W measured directly at the end of the fiber tip. He treated a second group of 10 samples with the laser set at 250 mJ, with an output power of 1.5 W. He used a pulse rate of 20 Hz for both groups. He treated each sample with one lasing cycle, which consisted of five irradiations of five seconds each with a 20-second break in between. For irradiation, he inserted the optical tip as far as the apex. Then he activated the laser and continuously radiated the root canal from the apex to the crown in slow, circular movements. By using this procedure, he could ensure that he irradiated the entire root canal. For each test microorganism, 10 samples served as a control group. He treated these control samples the same way he treated the test samples, but when he introduced the laser fiber into the canal, he did not activate the laser device.

Bacteriological evaluation. Immediately after the laser treatment we rinsed the root canal with 1 mL of physiological saline solution and collected the eluate in a microcentifuge tube.

To determine the bacterial count, we diluted the eluate in log 10 steps (that is, a dilution was made to the 10th part of the initial concentration, then to 100th part, the 1,000th part and so forth). We applied 20 µL of each dilution to sheep blood agar culture plates and incubated them for 24 hours at 37 C. We counted the colonies and assessed the total number of bacteria (CFU/mL of the extraction fluid). The lowest detection level of bacteria we found was 5×10^2 CFU/mL.

Temperature measurements. To assess the thermal impact of the irradiation using Er,Cr:YSGG lasers, we took temperature measurements, using five samples for each power setting. We mounted the teeth on an even thermocouple measuring 10 mm × 10 mm that used a silicon-based heat-conductive compound. During the irradiation procedure, which we conducted the same way as we did for the inoculated samples, we recorded the maximum temperature increase starting from a room temperature of 21 C by using a digital thermometer. We calculated the average value and the standard deviation of the five measurements from each laser and setting.

ESEM. We divided the remaining 20 samples in two equal groups (those irradiated with an output power of 1 W and of 1.5 W) and prepared them as described above, except we did not inoculate them with bacteria. We cut the samples longitudinally with a diamond-coated band saw and submitted them to SEM to evaluate the morphological changes induced by laser irradiation. We

TABLE

Bacterial counts of *Escherichia coli* and *Enterococcus faecalis*, by test group.

BACTERIA (LOG STEP OF COLONY COUNT)	TEST GROUP (NO.)		
	Control	Er,Cr:YSGG* (1 Watt)	Er,Cr:YSGG (1.5 W)
***Escherichia coli* (CFU/mL†)**			
Below detection level	—‡	8	10
10^3	—	—	—
10^4	—	2	—
10^5	5	—	—
10^6	5	—	—
10^7	—	—	—
***Enterococcus faecalis* (CFU/mL)**			
Below detection level	—	8	8
10^3	—	2	2
10^4	—	—	—
10^5	4	—	—
10^6	6	—	—
10^7	—	—	—

* Er,Cr:YSGG: Erbium, chromium:yttrium-scandium-gallium-garnet.
† CFU/mL: Colony-forming units per milliliter.
‡ —: Not applicable.

assessed the specimens using an ESEM, which works with comparatively small negative pressure compared with SEM, and the samples did not sputter, which facilitated our assessment of the samples and the minimization of artifacts. We took micrographs at different magnifications.

RESULTS

Bacteriology. The table shows the results of the bacteria counts of *E. coli* and *E. faecalis.* We rated samples in log steps of the colony counts and applied the specific radiation power.

The control group results for both test bacteria showed colony counts ranging between 10^5 and 10^6 CFU/mL, demonstrating a decrease of two to three log steps through the inoculation and incubation processes and the further processing of the samples.

The Er,Cr:YSGG laser succeeded in reducing the amount of *E. coli* at the lower output power setting of 1 W. The higher output power setting of 1.5 W yielded a reduction of the bacteria to below the detection level.

The Er,Cr:YSGG laser was effective in eliminating the gram-positive *E. faecalis.* At an output power of 1 W, it removed the bacterium three to four log steps compared with the control group. At an output power of 1.5 W, the use of the laser did not result in any significant difference in terms of disinfecting effectiveness when compared with the 1 W group.

Temperature measurements. When we chose the output power setting of 1 W, the irradiation of the samples resulted in an average temperature rise of 2.7 C at the root surface. When we chose the output power setting of 1.5 W, the average temperature rise at the root surface was 3.2 C. We took all the measurements at a base room temperature of 21 C; thus, the value 3.2 C stands for a temperature rise to 24.2 C. Although a higher irradiation power resulted in a stronger temperature increase at the sample surface, the temperature stayed within safe borders.

Environmental scanning electron microscopy. Figure 1 shows a longitudinal cut through a root that had been irradiated with the Er,Cr:YSGG laser at an output power of 1 W. The cut surface of the periluminal dentin can be seen at the right margin. The root canal surface exhibits the typical rough structure after the removal of the adhering smear layer.

Figure 2 gives a detailed view of the root canal wall at 1,000-fold magnification after irradiation with the laser set an output power of 1 W. The exposed dentinal tubules are clearly discernible, since the smear layer resulting from manual preparation had been removed by laser irradiation.

When we increased the output power to 1.5 W, we could see some areas with partially closed dentinal tubules (Figure 3, page 954). Partial melting and recrystallization of dentin could have caused this effect. A significant number of dentinal tubules stayed open.

Figure 4 (page 954) shows a detail of a root

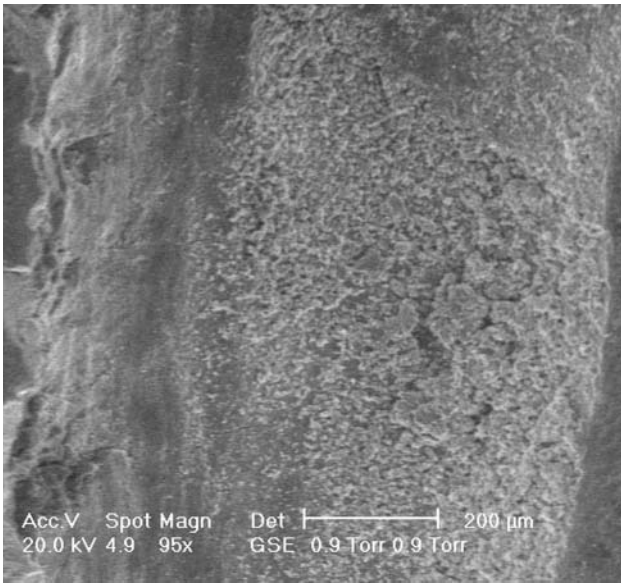

Figure. 1. Scanning electron micrograph of a root canal irradiated at an output power of 1 watt. Magnification ×95.

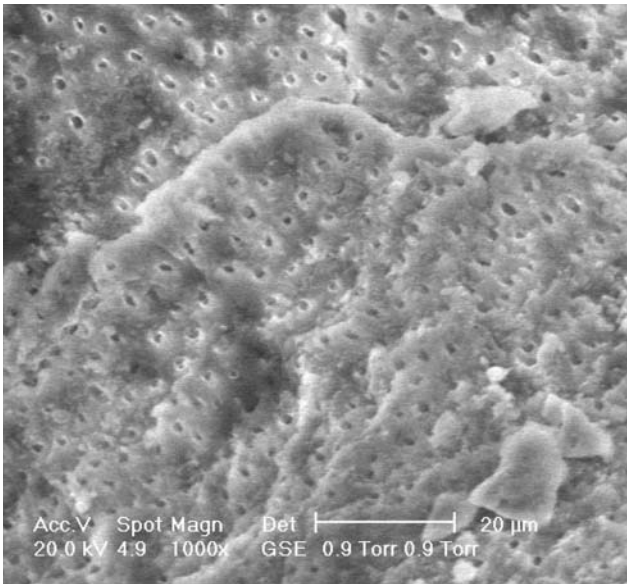

Figure 2. Scanning electron micrograph of a root canal irradiated at an output power of 1 watt. Magnification ×1,000.

canal irradiated at an output power of 1.5 W. The closed dentinal tubules can be seen alongside the open ones.

DISCUSSION

Successful endodontic treatment relies to a great extent on completely cleaning the root canal, as infected dentin and pulpal tissue can endanger therapy outcome. In conventional endodontic treatment, practitioners aim to remove infected pulp and dentin layers by using mechanical techniques and bactericidal irrigants. One treatment method, bactericidal rinsing, can be ineffective. Kouchi and colleagues[9] show that bacteria colonize the periluminal dentin up to a depth of 1,100 µm, while another study found that chemical disinfectants penetrate the dentin to a depth of only 100 µm.[10] Curved root canals or side branches also can be obstacles in conventional root canal treatment. The use of lasers helps overcome these problems. The high penetration depth of the laser beam in the dentinal tissue seems to be the best explanation of the satisfying bactericidal effect of different laser wavelengths. Vaarkamp and colleagues[15] and Odor and colleagues[16] provide a possible explanation for this kind of light propagation; they describe the ability of enamel prisms and dentin tubules to act as optical fibers. Moritz and colleagues[36] demonstrate the effect of Nd:YAG laser irradiation on bacteria through indirect irradiation.

The use of laser wavelengths suitable for the preparation of dental hard substances could add another aspect to the field of root canal cleaning. In an in vitro study, Schoop and colleagues[29] described the effects of Er:YAG laser irradiation on root canal walls and the bactericidal effect of its wavelength, which emits at 2,940 nm.

We conducted our study to evaluate the effectiveness of a similar wavelength, that of the Er,Cr:YSGG laser, which emits at 2,780 nm. Even at the lower output power of 1 W, we observed a distinct reduction in bacterial counts, although we could not achieve bacterial reduction below 10^2 CFU/mL in all the samples. When we used the laser at an output power setting of 1.5 W, the CFU/mL level fell below the detection level in all samples. We found no significant difference between the effects of laser irradiation on the two test microorganisms. In another study, the authors stated that *E. faecalis* was harder to eradicate than *E. coli* and explained that this was due to differences in cell wall structures.[36] In a preceding study that focused on the Er:YAG laser, the investigators noticed no complete eradication of *E. faecalis*.[30]

The temperature rise at the root surface did not exceed 3.2 C; therefore, we discounted excessive heating of the entire sample as a reason for the antibacterial effect. We also regarded the maximum temperature rise at the root surface as innocuous for the surrounding periodontal tissues.

Owing to the high absorption rate of the Er,Cr:YSGG laser's radiation in water, the penetration depth in dentin should be restricted,

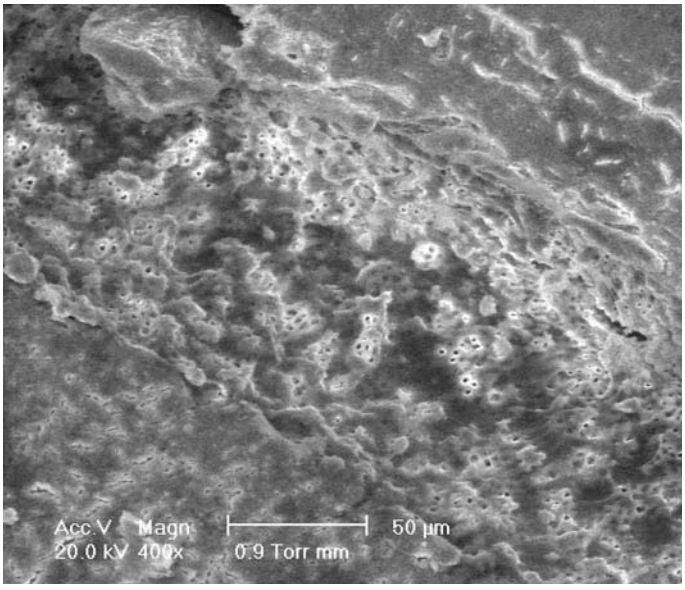

Figure 3. Scanning electron micrograph of a root canal irradiated at an output power of 1.5 watts. Magnification ×400.

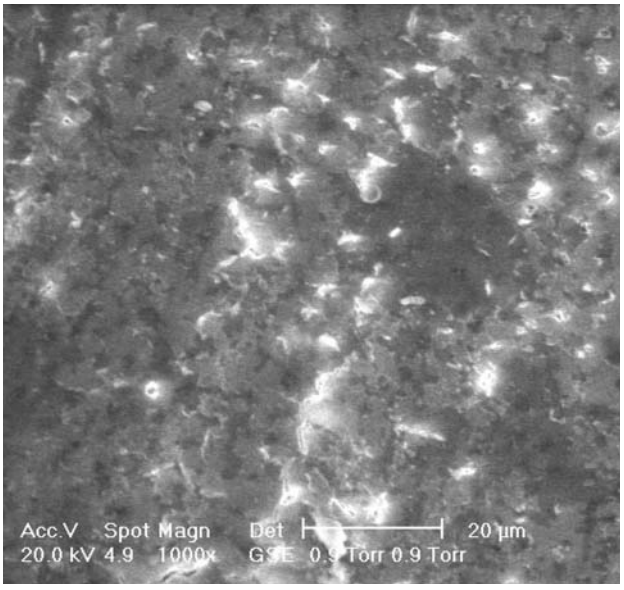

Figure 4. Scanning electron micrograph of a root canal irradiated at an output power of 1.5 watts. Magnification ×1,000.

unlike with other lasers such as the Nd:YAG or the diode. One explanation for the laser's positive bactericidal effect on *E. faecalis* in our study could be that the test bacteria did not penetrate the dentinal tubules. However, we incubated the samples for four hours before laser irradiation, which should have been sufficient to allow for propagation of the test bacteria into the tubular network. This method's reliability has been demonstrated in other studies.[24,30,36] Another explanation for the Er,Cr:YSGG laser's positive effect on *E. faecalis* could be that a certain degree of the laser's light conduction within the dentinal tubules results in a higher penetration depth. Other factors such as shock waves or cavitation effects have been reported for other laser devices[37,38] and could be a further explanation for the actual effects of Er,Cr:YSGG laser irradiation. Further investigations are necessary to clarify the exact interactions of laser light and bacteria.

Through SEM, we found that the Er,Cr:YSGG laser could remove the smear layer and debris from the root canals' walls and could open up the dentinal tubules' orifices. This should help practitioners seal the root canal tightly. We used the laser as an adjunct to the conventional root canal preparation technique. Although we did not observe excessive ablation, we think that using the laser to enlarge the canal could lead to unwanted effects such as perforating the root canal wall or local overheating.

When we used the laser at the higher output power setting (1.5 W), we found slight traces of melting and recrystallization. This might be due to the fact that we used the laser without using the air-water delivery system so as not to impair our bacteriological evaluation. Since the device is equipped with an adjustable air-water delivery system, using the water spray probably could reduce those effects. We are conducting a study on the effects of the water spray and the rinsing solutions on the laser's effectiveness.

Regarding the ability of the Er,Cr:YSGG laser system to remove debris and the smear layer from the root canal walls and to reduce the presence of viable bacteria, we found that it yielded results equivalent to those of lasers with different wavelengths.[17-36]

CONCLUSION

The laser wavelength we tested in our study may be suitable for cleaning and disinfecting root canals and can be used safely if the common precautions for using lasers are observed and the energy and irradiation time are within the proposed range. Clinical studies are necessary to confirm the results and to investigate the laser wavelengths under in vivo conditions. ■

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