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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*

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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.

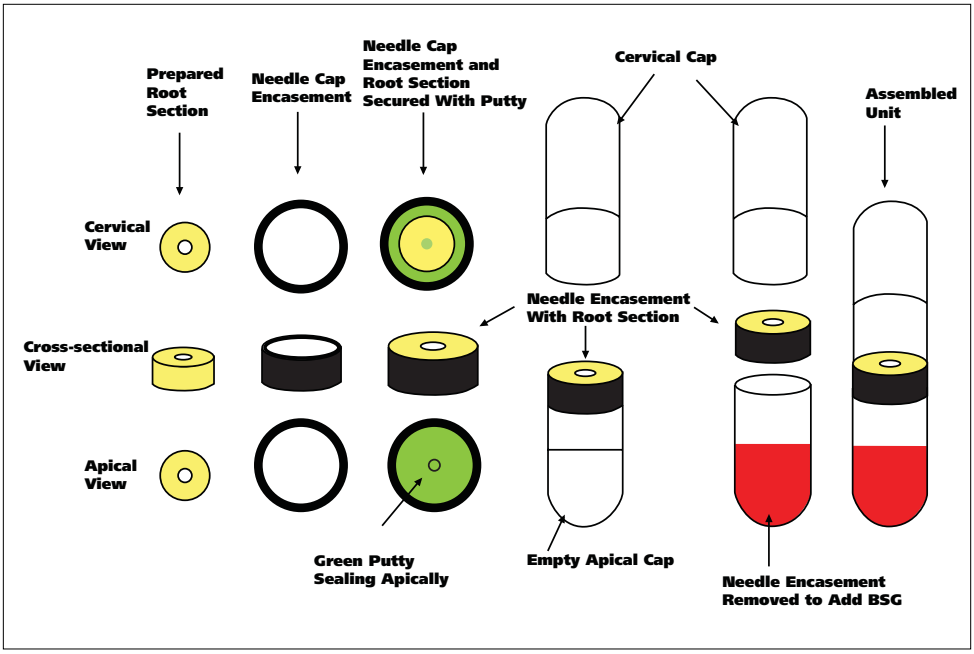

Figure 1. Model preparation. Buffered saline with gelatin (BSG) contains 8.5 grams sodium chloride, 0.3 g anhydrous monopotassium phosphate, 0.6 g anhydrous disodium phosphate and 0.1 g of gelatin per liter of distilled water.

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

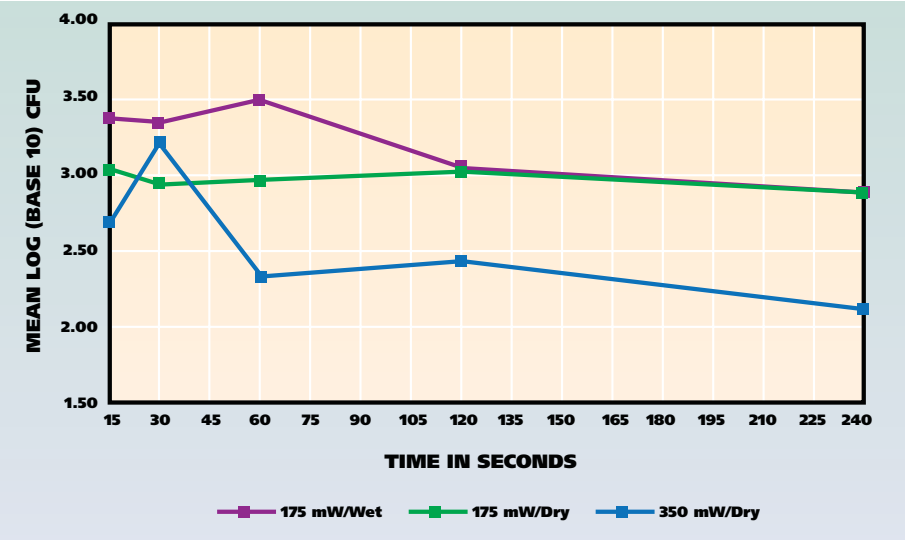

Figure 3. Plot of mean logarithmic scale colony-forming units (log CFUs) over time for the three laser treatment conditions. mW: Milliwatts.

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.

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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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