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## Immediate root canal disinfection with ultraviolet light: an ex vivo feasibility study

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**Objective.** The study was designed to test application of ultraviolet light to root canal walls, as a mean of complementary immediate disinfection after the use of sodium hypochlorite.

**Study design.** Root canals were infected ex vivo with *Enterococcus faecalis* for 48 hours. Nonattached bacteria were washed away, and the remaining attached bacteria were subjected to disinfection, with 5% sodium hypochlorite alone or followed by exposure to ultraviolet light (254 nm, 300 mJ/cm<sup>2</sup>). Root canals were then tested for remaining viable bacteria. Canals were obturated and tested again after 14 days.

**Results.** Sodium hypochlorite alone achieved negative cultures in only 47% of the cases, but 96% was achieved with sodium hypochlorite followed by ultraviolet light ( $P < .001$ ). This status was also maintained after 14 days.

**Conclusions.** Illumination of root canals with ultraviolet light may be an effective supplementary means to achieve immediate disinfection of infected root canals. (*Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:425-33)

Effective disinfection is the initial and fundamental goal of root canal treatment of infected root canals. Sodium hypochlorite (NaOCl) alone fails to achieve this goal in many cases.<sup>1</sup> This has been further confirmed by several groups of investigators.<sup>2-6</sup> Siqueira et al. tested the effect of 4% NaOCl applied for 5 minutes in *Enterococcus faecalis*-infected root canals in vitro and concluded that 40% of the root canals still harbored viable bacteria after the treatment.<sup>2</sup> Sjögren et al. reported that clinical application of 0.5% NaOCl failed to eliminate all bacteria in infected root canals and that 40% had positive cultures at the end of the procedure.<sup>3</sup> Shuping et al., in a clinical study, tested the effect of extensive apical enlargement combined with 1.25% NaOCl and concluded that negative cultures were obtained from only 62% of the root canals and that 38% remained positive.<sup>4</sup> Similar results have recently been reported by Shabahang and Torabinejad, who applied 5.25% NaOCl for ~5 minutes to *E. faecalis*-infected root canals in vitro. Negative cultures

were achieved in only 47% of the cases.<sup>5</sup> Even a prolonged 30-minute application of 5.25% NaOCl failed to render all root canals bacteria free.<sup>6</sup> Therefore, it can be concluded that NaOCl frequently fails to achieve the desired goal of root canal treatment: a bacteria-free root canal.

This limited ability of NaOCl calls for application of additional measures to render an infected root canal free of viable bacteria. Intracanal medication with calcium hydroxide [Ca(OH)<sub>2</sub>] is commonly applied for 1 to 2 weeks.<sup>7</sup> This procedure may result in up to 93% negative cultures.<sup>4</sup> Nevertheless, it often requires several applications to reach that goal.<sup>8</sup> This protocol has 3 major drawbacks: It is time consuming, a single application may be unpredictable,<sup>8,9</sup> and resistant strains may grow in the medicated root canal.<sup>8</sup>

Recently, 1-visit endodontic protocols have gained in popularity, but application of these protocols in infected root canals is still controversial.<sup>10</sup> Attempts to achieve a better disinfection have led to the introduction of a final rinse, with solutions containing either chlorhexidine or doxycycline.<sup>5,11,12</sup> The effectiveness of both may be partially attributed to a residual antibacterial agent that binds to the radicular dentin, and the long-term effects of their presence have not yet been studied.

In search for a more effective disinfectant agent, the potential of ultraviolet (UV) light to eliminate root canal bacteria has been considered. Ultraviolet light is widely used for disinfection of surgical and laboratory surfaces, for water disinfection, and disinfection of the outer surface of fruits. In a recent preliminary study,<sup>13</sup> it was established that oral bacteria commonly found in

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infected root canals are highly sensitive to 254 nm UV light. When directly exposed, doses as low as 7 mJ/cm<sup>2</sup> were sufficient to eliminate all bacterial strains tested, including a Ca(OH)<sub>2</sub>-resistant strain of *E. faecalis*. However, when bacteria in a multilayer were exposed, much higher doses were required to compensate for absorption of UV light by the outer layers of bacteria.<sup>13</sup> This may indicate that if effective use of this agent in root canals is considered, most of the bacteria and tissue remnants should first be removed by a preliminary mechanical cleaning and application of agents, such as NaOCl.

The present study was designed as an ex vivo feasibility study to examine the potential of intracanal UV light application as a supplementary root canal disinfecting measure, to be used after the use of NaOCl.

## MATERIALS AND METHODS

### Teeth

From a random collection of teeth, extracted for periodontal or prosthodontic reasons, freshly extracted, single-rooted teeth with no previous endodontic treatment were selected. Teeth were stored at 4°C and 100% humidity, with no antiseptic, until used.

### Root canal preparation

Teeth were horizontally cut at the cemento-enamel junction, using a high-speed diamond bur cooled with air-water spray. Root canals were endodontically prepared using the ProTaper rotary file sequence up to no. F3 (Dentsply Maillefer, Ballaigues, Switzerland). Working solutions of 6% NaOCl (Bio Lab, Jerusalem, Israel) and 18% EDTA (Ultradent, South Jordan, UT) were alternately used after each instrument. Following the ProTaper instrumentation, the apical part of the canal was further prepared manually to size 50 using K files (Dentsply Maillefer). A final application of 18% EDTA was used to remove potential remnants of smear layer.

Each prepared root canal was measured and the root coronally reduced to a uniform root canal length of 13 mm in all roots. Each canal was carefully inspected at ×8 magnification, and canals with any abnormalities were rejected; only root canals with a regular preparation and a round cross-section were included. Each apical foramen was sealed with an epoxy resin applied on the outer surface of the apex. A master cone, prefitted in the apical part of the canal, prevented the resin from entering the canal lumen. The outer root surface was sealed with a double coat of nail varnish. Roots were embedded, apices down, in plaster cylinders to a level 3 mm short of the cut surface. The root canal space was measured volumetrically and the mean volume was 10 (±1.2) µL. All roots were autoclaved and

stored at 4°C and 100% humidity until used. From this point, strict asepsis was applied, and all procedures were carried out in a bacteriologic hood.

### Bacteria

*Enterococcus faecalis* TA was used to infect the root canals. This strain is a clinical isolate, initially isolated and previously used by Weiss et al.,<sup>14</sup> and stored as a frozen stock in our laboratory. Exposure of this strain to 254-nm UV light at 2 mJ/cm<sup>2</sup> resulted in 99.9% killing.<sup>13</sup> Bacteria were grown aerobically at 37°C in brain-heart infusion broth (Difco; Becton Dickinson, Sparks, MD).

Sterile broth, 5 mL, was inoculated with 200 µL of an overnight-grown *E. faecalis* culture containing 1 × 10<sup>9</sup> bacteria per mL. Each root canal was filled with the freshly inoculated broth and incubated at 37°C and 100% humidity for 24 hours. At this time, the canal content was gently replaced with a fresh, similarly inoculated, culture broth and incubated for an additional 24 hours. Thus, *E. faecalis* was allowed to grow in the root canals for 48 hours. Distel et al. recently demonstrated that under similar conditions *E. faecalis* totally colonized the root canal walls.<sup>15</sup>

### Experimental design

Root canals with 48-hour *E. faecalis* growth were washed to remove all unattached bacteria. The remaining attached (nonwashable) bacteria were subjected to disinfection (stage a) by either NaOCl alone (control) or by NaOCl followed by UV light (experimental), and the canals were sampled for viable bacteria. Root canals were then obturated (stage b). After 14 days of incubation, the root canal fillings were aseptically removed and the bacterial content of the canals sampled again and compared with the postdisinfection/preobturation status.

### Unattached bacteria control

At 48 hours after inoculation, the washable content of each root canal was washed and harvested with sterile saline. A 1-mL tuberculin syringe, adapted with a sterile 25-gauge needle, was filled with 100 µL sterile saline. The needle was inserted freely to the apex, and the content of the root canal was aspirated into the syringe. The root canal was refilled from this syringe and the content aspirated. This was repeated 3 times, resulting in 110 µL of bacterial suspension, which was then added to 890 µL sterile saline (total 1 mL). Samples were serially diluted to 1:10. A 10-µL sample of each dilution was streaked on BHI agar (Hy Laboratories, Rehovot, Israel) and grown aerobically at 37°C for 24 hours, when colonies with typical *E. faecalis* morphology could clearly be seen, distinguished, and

counted. These samples of unattached bacteria were harvested from each infected root canal before each experiment to verify uniform bacterial growth of the inoculum. They represented the unattached (washable) bacterial content of the canal.

### Colony counting

To allow both data recording and easy accurate counting of colony-forming units (CFU), digital photographs of the plates were taken, transferred to a computer, and adapted with a digital grid. Digital enlargement by  $\times 5$  allowed CFU to be easily counted from the screen and the bacterial content of the sample to be calculated.

### Attached bacteria control

In preliminary experiments, the remaining, nonwashable, bacterial content of the root canals was also determined. Infected root canals ( $n = 20$ ) were washed as above, followed by sampling of the attached bacteria remaining in the canal after washing, using a modification of the method used by McGurkin-Smith et al.<sup>6</sup> Bacteria were resuspended into a saline sample of known volume. A 1-mL tuberculin syringe, containing 100  $\mu\text{L}$  sterile saline and adapted with a thin (25-gauge) needle was used to fill the root canal just short of its ream. A slightly bent no. 30 sterile K file was inserted into the canal and gently moved in and out with a circular motion. The instrument gently touched the canal walls to resuspend the attached bacteria, while taking care not to file the dentin.

The canal content was aspirated into the syringe and the procedure repeated twice. This resulted in a 100- $\mu\text{L}$  sample containing bacteria released from the pool of attached bacteria that remained in the root canal after the initial rinsing step mentioned above.

The harvesting method was a modification of the method used by McGurkin-Smith et al.<sup>6</sup> They also used a fluid-filled canal in which a sterile file, identical to the final file used in preparation, was pumped in and out to suspend remaining bacteria. They collected the fluid by absorbing it into a sterile paper point. This method was modified as described here to reduce the chance of filing the canal walls when using a thick and rather rigid file and to generate an immediate direct suspension of all harvested bacteria rather than attempting to resuspend them later from the paper point.

The harvested bacteria represented the bacterial biomass remaining in the root canal after the initial saline wash. This attached bacterial biomass was the actual target for the disinfection experiments that would follow. The above procedure was carried out as a preliminary control in 20 root canals. It quantitatively established the reproducible presence of attached bacteria

after 48-hour growth and washing off the unattached content of the canal with saline. Naturally, this step was not performed in each disinfection experiment, because the undisturbed bacterial layer had to remain in place as the target of the disinfection agents used.

### Sampling procedures in experimental stages a and b

The bacterial content of root canals in the experimental and control groups of stage a was sampled after exposure to either NaOCl + UV light or NaOCl alone, respectively, applying the method used to harvest attached bacteria described in the preceding. Either sterile saline or sterile saline containing 6% sodium thiosulphate was used for harvesting. The latter was applied when the sampling was immediately after exposure to NaOCl, as detailed subsequently. Sodium thiosulphate alone, at the concentrations used, had no effect on bacterial viability or growth (data not presented). Bacterial content of root canals after removal of root canal fillings (stage b) was sampled by the same methods as in stage a.

### Stage a: Disinfection of *E. faecalis*-infected root canals

*Enterococcus faecalis*-infected root canals were disinfected with either 5% NaOCl followed by an UV exposure of 300  $\text{mJ}/\text{cm}^2$  (experimental group, 25 root canals), or by 5% NaOCl alone (control group, 30 root canals), as described subsequently.

*Control group: NaOCl alone.* Root canals in which *E. faecalis* grew for 48 hours were first washed with saline to remove any free-floating and washable bacteria. Washed bacteria from each canal were collected and handled as already described to determine positive and uniform growth of each inoculum. The saline was washed out and replaced with 5% NaOCl. The solution remained in the canal for 5 minutes, then was replaced with fresh 5% NaOCl for an additional 5 minutes. At the end of the 10-minute exposure, NaOCl was replaced with 6% sodium thiosulphate solution, which was refreshed after 5 minutes and maintained in the canal for an additional 5 minutes to neutralize the NaOCl.<sup>4,16</sup> The root canals were then dried with sterile paper points. All of these procedures were carried out under strict asepsis in a bacteriologic hood.

In experiments that included NaOCl alone, sampling of the remaining viable bacteria was done at this stage, as already described.

*Experimental group: NaOCl + UV light.* All root canals were initially treated with NaOCl, as in the control group. After drying with sterile paper points, canals were exposed to UV light (300  $\text{mJ}/\text{cm}^2$ , 240-second exposure, with the UV light source below).

Sampling of viable bacteria after this additional treatment was as already described. The control samples were kept for the same time at the same conditions and assayed together with the experimental samples.

**Root canal anatomy control.** To control for a potential effect that variations in root canal anatomy and/or preparation may have on the results, 14 root canals were treated with both disinfection protocols, successively. Canals were first disinfected with NaOCl followed by its neutralization with sodium thiosulphate and sampled. The same canals were then reinfected for 48 hours, washed with saline, disinfected again with NaOCl + UV light, and sampled. Sampling of remaining viable bacteria was carried out after each stage, as already described.

### Stage b: Effect of root canal fillings on the bacteriologic status

Root canal fillings were placed (as described subsequently) in 30 of the root canals included in either of the stage a disinfection groups. Initially, 15 were treated with NaOCl + UV light (experimental group) and the other 15 were treated with NaOCl alone (control group). All filled roots were incubated for 14 days in a closed box at 37°C and 100% humidity. The fillings were then aseptically removed and the root canals sampled. The bacteriologic status of each root canal, as determined after incubation was compared with that determined before obturation.

**Root canal obturation and reopening.** Root canal obturation was carried out by lateral condensation, using RoekoSeal sealer (Roeko, Langenau, Germany) and gutta-percha. We initially established that this sealer had no antimicrobial effect (data not presented), which is in agreement with the manufacturer's statements. Upon completion of lateral condensation, excess gutta-percha was removed with a red-hot instrument to the level of the cut root surface. An additional coronal seal was added by covering the cut surface with a thick layer of RoekoSeal sealer that could later be easily and cleanly removed with no drilling and leaving no remnants on the cut root surface.

After completion of this procedure, roots were stored in a closed box at 37°C and 100% humidity for 14 days. At the end of this period, the coronal seal was aseptically removed and the cut root surface disinfected by UV exposure (300 mJ/cm<sup>2</sup>). Root canal fillings were then aseptically removed by inserting a sterile no. 40 Hedström file into the center of the root canal filling mass, using a clockwise movement, to a depth of 5-6 mm, followed by gentle pulling. This method enabled removal of the root canal filling as one mass. The removed filling was inspected under ×8 magnification to verify that it was all removed and no parts were

missing. In a few cases in which one of the accessory cones remained in the canal, it was gently removed using a sterile Hedström file. The root canal bacterial content was sampled as already described. All procedures were performed in strict asepsis in a bacteriologic hood.

### UV light source and delivery

A mercury lamp (Oriol Instruments, Stamford, CT), used as the source of UV light, was adapted with a fiber-optic line (Polymicro Technologies, Phoenix, AZ) and a coupler that attached it to a specially designed intracanal UV diffuser (InterLight, Or-Yehuda, Israel). The diffuser had a cylindroconic shape with dimensions that allowed its insertion up to the apical end of each root canal ( $D_0 = 0.25$  mm;  $D_{12} = 0.8$  mm). It allowed a uniform circumferential illumination with light emission perpendicular to its surface. Because the bactericidal effect of UV light peaks at a wavelength of 254 nm,<sup>17</sup> the mercury lamp, which has a broader spectrum, was fitted with a filter (Andover Corporation, Salem, NH), resulting in a narrow spectrum of 250 to 270 nm, with a peak at 260 nm. A photometer (Ophir Optronics, Jerusalem, Israel), calibrated to measure only at the wave length of 254 nm, was used in each experiment to measure the actual energy flow at this wavelength at the surfaces of the UV intracanal diffuser. Exposure times were calculated to allow 300 mJ/cm<sup>2</sup> of exposure in the root canals (240 seconds with this setup).

### UV passage through dentin

To evaluate the passage of UV light through dentin, thin slices of dentin were prepared, ranging in thickness from 80 μm to 200 μm. The slices were prepared in a way that the dentinal tubules were perpendicular to the surface of the slice. Slices were illuminated with UV light from one side. Light passing through the dentin slice was measured on the other side, using a photometer calibrated to a wavelength of 254 nm. A quartz filter 500 μm thick was used as a positive control, and a regular glass slide 500 μm thick was used as a negative control.

### Statistical analysis

Fisher exact test was used to compare the incidence of positive cultures between the groups treated with NaOCl alone and those treated by a combination of NaOCl + UV. McNemar Test was used to compare the bacteriologic status of root canals before and after 14 days with a root canal filling.

**Table I.** Disinfection with NaOCl alone compared with NaOCl + UV

	Culture	
	Positive	Negative
5% NaOCl	53% (16/30)	47% (14/30)
5% NaOCl + UV	4% (1/25)	96% (24/30)

Total sample: 30 root canals with NaOCl alone, 25 with NaOCl + UV. NaOCl (5%) refreshed after 5 min, applied for a total of 10 min, then neutralized with 6% sodium thiosulphate. UV light (254 nm) applied through a diffuser at 300 mJ/cm<sup>2</sup> after NaOCl disinfection.

**RESULTS**

**Inoculation and rinsing**

The method used for inoculation resulted in uniform bacterial growth in all root canals. The bacterial content harvested in the initial saline rinsing of unattached bacteria yielded an average of  $9.5 \times 10^6 (\pm 0.97 \times 10^6)$  CFU. In a root canal with a volume of 10  $\mu$ L, this yield was equivalent to  $9.5 \times 10^8 (\pm 0.97 \times 10^8)$  CFU/mL.

Harvesting the bacteria remaining in the root canal after the initial saline rinse, using the mechanical suspension method with a bent no. 30 file, yielded an average of  $8.5 \times 10^4 (\pm 1.5 \times 10^4)$  CFU. This consistent and uniform yield demonstrates that the sampling procedure used in the present study is an effective and reproducible way to sample bacteria attached to the canal walls. The collected sample represents a bacterial count less than or equal to the bacterial target of the disinfection procedures applied in the following experiments.

**Control: Disinfection with 5% NaOCl alone**

A limited disinfecting ability was found when NaOCl was used alone. In 14 of the 30 samples, viable bacteria still remained in the canals (Table I). The goal of effectively disinfecting the root canals was attained in only 47% of the samples.

**Experimental: Disinfection with 5% NaOCl + UV light**

No viable bacteria were found in 24 of the 25 samples when NaOCl was supplemented with UV treatment at 300 mJ/cm<sup>2</sup> (Table I). The goal of effectively disinfecting the root canals was attained in 96% of the samples, which was statistically significant compared with NaOCl alone ( $P < .001$ ).

**Root canal anatomy control**

Results were similar to the experimental and control groups when both protocols were tested sequentially in the same root canals. The NaOCl alone failed to eliminate all viable bacteria from the infected root canal in

**Table II.** Root canal anatomy control: Disinfection with NaOCl alone compared with NaOCl + UV, when applied successively in the same root canals

	Culture	
	Positive	Negative
5% NaOCl	50% (7/14)	50% (7/14)
5% NaOCl + UV	7% (1/14)	93% (13/14)

A total of 14 teeth were first disinfected with NaOCl alone, then reinfected and disinfected with NaOCl + UV. The 5% NaOCl was refreshed after 5 min, applied for a total of 10 min, then neutralized with 6% sodium thiosulphate. The UV light (254 nm) was applied by a diffuser at 300 mJ/cm<sup>2</sup>, after an initial NaOCl disinfection.

**Table III.** Bacteriologic status of root canals before and after 14 days with a root canal filling

	Culture			
	Positive		Negative	
	Before	After	Before	After
NaOCl	60% (9/15)	60% (9/15)	40% (6/15)	40% (6/15)
NaOCl + UV	0% (0/15)	13% (2/15)	100% (15/15)	87% (13/15)

Microbiologic status determined immediately after disinfection (before obturation) and after 14 days and removal of the root canal filling. 5% NaOCl refreshed after 5 min, applied for a total of 10 min, then neutralized with 6% sodium thiosulphate. UV light (254 nm) applied by a diffuser at 300 mJ/cm<sup>2</sup>, after an initial NaOCl disinfection.

7 of the 14 root canals (50%; Table II). When the same root canals were reinfected and tested with NaOCl supplemented with UV light treatment, the desired result was achieved in 13 of the same 14 root canals (93%; Table II;  $P < .03$ ). It should be noted that the initial harvesting of the washable bacterial canal content with saline before the second treatment confirmed that the first treatment did not affect bacterial growth in these root canals in the second stage (data not presented).

**Bacteriologic status before and after 14 days with a root canal filling**

The microbiologic status of the root canals after removal of the root canal fillings was compared with that recorded immediately after disinfection and before root canal obturation.

Root canals in the group initially disinfected with NaOCl alone yielded positive cultures in 60% of the cases before obturation (Table III). Similarly, these root canals also yielded 60% positive cultures after removal of the root canal fillings. Within this group, 2 initially negative root canals were positive after 14 days and 1 initially positive canal was negative after 14 days. Nevertheless, no significant difference was found (Mc-

**Table IV.** Passage of UV light through dentin of varying thickness

Sample	Thickness	UV light passing
Quartz control	500 $\mu\text{m}$	100%
Glass control	500 $\mu\text{m}$	0%
Dentin	80 $\mu\text{m}$	2.5%
Dentin	100 $\mu\text{m}$	1.0%
Dentin	150 $\mu\text{m}$	0.1%
Dentin	180 $\mu\text{m}$	0.025%
Dentin	200 $\mu\text{m}$	0%

Mean percentage of UV light passing through the sample. SEM were within 10% of the mean.

Nemar test) between the bacteriologic condition before and after 14 days.

All 15 root canals in the group treated with the combined protocol of NaOCl + UV yielded no viable bacteria before root canal filling. The same microbiologic status was found in 13 of these canals when reopened after 14 days (no significant difference between before and after).

#### Passage of UV light through dentin

A 0.5-mm thick glass slide absorbed 100% of the 254 nm UV light, and a similar thickness of quartz allowed passage of 100% of the light (Table IV). Thick dentin layers (200  $\mu\text{m}$ ) absorbed 100% of the UV light, and the thinnest layer (80  $\mu\text{m}$ ) allowed passage of only 2.5% of the UV light. A dentin layer of 100  $\mu\text{m}$  allowed passage of 1% of the light. This was used as a reference to calculate the UV dose of 300  $\text{mJ}/\text{cm}^2$ , as discussed subsequently.

#### DISCUSSION

Sodium hypochlorite is an extremely effective disinfectant when applied to free-floating ("planktonic") bacteria. Recently, Gomes et al. found that 5.25% NaOCl eliminated 100% of the free-floating *E. faecalis* bacteria in less than 30 seconds.<sup>16</sup> Nevertheless, when tested in infected root canals, NaOCl failed to render them free of viable bacteria.<sup>1-6</sup> Incidence of positive cultures after application of NaOCl alone in these studies ranged from 38% to 52%.

In the present study, 53% of the infected root canals that were exposed to 5% NaOCl for 10 minutes still harbored viable bacteria, which is in agreement with others.<sup>1-6</sup> It may be possible that more extended exposure times or heating the solution may result in a better outcome, but that is beyond the scope of the present preliminary study.

The finding that NaOCl is not as effective a disinfectant as is commonly believed calls for additional measures to achieve the desired disinfection goal of

root canal treatment. Intracanal application of  $\text{Ca}(\text{OH})_2$  between appointments has been widely advocated.<sup>7,10</sup> In a clinical study, application of  $\text{Ca}(\text{OH})_2$  for 1 week resulted in negative cultures in 92% of the cases, compared with 62% when only NaOCl was used.<sup>4</sup>

Nevertheless,  $\text{Ca}(\text{OH})_2$  is not the ultimate answer to root canal disinfection. Peters et al. have reported that when used as an interappointment dressing it failed to prevent an increase in the number of root canals that yielded positive cultures.<sup>18</sup> A recent report further demonstrated the unpredictability in the clinical application of the  $\text{Ca}(\text{OH})_2$  protocol. A single application of  $\text{Ca}(\text{OH})_2$  resulted in negative cultures in only 43% of the cases. A second application reached 87%, and three applications 100%.<sup>8</sup> Similar results pointing out the limited efficacy of  $\text{Ca}(\text{OH})_2$  were recently reported also by Waltimo et al.<sup>19</sup>

Resistant bacterial strains may partially explain these findings. It has been established that  $\text{Ca}(\text{OH})_2$ -resistant strains of *E. faecalis* are associated with endodontic failures in  $\text{Ca}(\text{OH})_2$ -treated root canals.<sup>20,21</sup> This phenomenon is not limited to enterococci alone, as lactobacilli and nonmutans streptococci may also be resistant to this intracanal dressing.<sup>8</sup>

Furthermore, *E. faecalis* can grow on the walls of root canals even while medicated with  $\text{Ca}(\text{OH})_2$ .<sup>15</sup> These drawbacks, taken together with the fact that interappointment dressing with  $\text{Ca}(\text{OH})_2$  is a time-consuming protocol, call for alternative, more effective, and immediate root canal disinfection methods.

Ultraviolet light has been widely used to disinfect drinking water and surfaces in operating rooms and laboratories and in other similar applications. Recently it has been reported that oral bacteria commonly found in infected root canals are highly sensitive to 254-nm UV light.<sup>13</sup> Doses as low as 7  $\text{mJ}/\text{cm}^2$  were sufficient to eliminate all bacteria of the strains tested, including a  $\text{Ca}(\text{OH})_2$ -resistant strain of *E. faecalis*.<sup>13</sup> Taken together with the results of the present study, UV light may present a potential method to achieve better root canal disinfection than NaOCl can provide and without the need for an interappointment dressing and a resulting second visit.

Nevertheless, before considering UV application for root canal disinfection, several issues must be addressed: 1) an effective method for its application to the canal walls; 2) the limited penetration of UV light through tissues and bacterial biomass; and 3) safety to the surrounding tissues.

The special diffuser used in the present study was an effective tool for UV light application to the canal walls and may be a partial answer to the first issue. However, a better UV light source must be developed, because mercury lamps, such as the one used, are cumbersome and relatively inefficient. It required 240 seconds to

deliver the desired dose in the present study which is a relatively long time for clinical application. Ultraviolet lasers could be a potential efficient source that may reduce exposure times to a few seconds, but they are, at present, too expensive for routine use. However, recent developments in UV light-emitting diodes (LEDs) may provide a cost-efficient answer to this problem.

Ultraviolet light has low penetration through a biomass, and when passing through a bacterial multilayer the outer bacteria may significantly absorb the 254-nm light.<sup>13</sup> This dictates that UV light should not be considered as a sole means of disinfection in a heavily contaminated environment such as the root canal. Initial reduction of the biomass by other means, such as mechanical debridement combined with NaOCl, is mandatory for the UV light to be effective. The UV light cannot be considered as a substitute for NaOCl. Therefore, in the present study UV light was not tested as a sole disinfection agent but as a supplementary means after application of 5% NaOCl that had 10 minutes to attack the bacterial biomass. As such, the UV light was quite effective. The NaOCl failed to eliminate all detectable viable bacteria in 53% of the canals, whereas addition of UV light achieved this goal in more than 90% of the canals.

In the present study, a UV dose of 300 mJ/cm<sup>2</sup> was selected on the basis of safety considerations, as will be discussed subsequently, and found to be effective. Further studies will be required to establish the lowest effective UV dose. Additionally, sensitivity of *E. faecalis* to other antimicrobial agents has been shown to be dependent on the age of the culture, with "starved" cultures being more resistant than young fast-growing ones.<sup>22</sup> Therefore, the present study should also be repeated with older "starved" bacteria in the root canals to verify whether similar results will be obtained.

To control for the possibility that variations in root canal anatomy may have contributed to the differences found, the experiment was repeated using the same roots for both protocols. The almost identical results (Table II) indicate that anatomic variations were not the source of the difference between the results of the 2 protocols.

The term disinfection rather than sterilization was preferred, throughout the present report, to indicate a substantial reduction in the number of viable bacteria in the root canal down to amounts below the detection level of the assay used. Furthermore, it expresses the understanding that the UV light cannot be expected to eliminate all bacteria in the dentinal tubules, because its penetration into dentin is limited. In that aspect, UV light is similar to Ca(OH)<sub>2</sub>. Nevertheless UV light has two major benefits over the latter: 1) Bacterial elimination may be instant, with no need for prolonged

dressing; and 2) UV light may be effective against a variety of bacterial strains, including those resistant to Ca(OH)<sub>2</sub>.<sup>13</sup>

The significance of bacteria remaining in the dentinal tubules is a widely discussed issue. Some refer to such bacteria as a major issue,<sup>5</sup> whereas others question their clinical significance.<sup>23,24</sup> Peters et al. have questioned the need for intratubular bacterial elimination and concluded that once the root canal itself was disinfected, a sound obturation technique will prevent the bacteria remaining in the tubules from repopulating the root canal space.<sup>23</sup> In the present study, to examine the postobturation dynamics of bacterial growth, root canals were sealed with an inert sealer and followed for 14 days. This was done because bacterial regrowth from the dentinal tubules may jeopardize the long-term effect of the disinfection.

No significant bacterial regrowth was found in disinfected and obturated root canals after 14 days of incubation, which is in apparent agreement with the ideas of Peters et al.<sup>23</sup> and Peters and Wesselink,<sup>25</sup> at least in the short range of 14 days. Nevertheless, because the present study did not demonstrate that dentinal tubules did contain viable bacteria after 48 hours of growth, such conclusions will have to be postponed until this part of the study is repeated with a protocol that will ensure and demonstrate bacterial invasion of dentinal tubules.

The model of "removable root canal filling" used in the present study may serve as an extremely useful tool to determine the role of root canal fillings in preventing regrowth of bacteria in the root canal. Obturated root canals were re-entered with no drilling, heating, or use of solvents. To the best of our knowledge, this model of "removable root canal filling" has never been used. Studies to determine the ability of such root canal fillings to prevent bacterial regrowth for longer periods of time are currently in progress.

The bactericidal mechanism of UV light is based on induction of DNA dysfunction caused by cross-linking between neighboring pyrimidine nucleotide bases (thymine and cytosine) in the same DNA strand. This in turn impairs formation of hydrogen bonds with the purine bases on the opposite strand. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death.<sup>26,27</sup> The same mechanism may potentially be harmful to the host cells. Therefore, certain precautions should be seriously considered before this protocol may be clinically applied.

The passage of UV light through dentin is limited and a dentin layer of 100 μm allows passage of only 1% of the light. The value of 300 mJ/cm<sup>2</sup> used in the present study was selected on the basis of this obser-

vation: If a minimal layer of 100  $\mu\text{m}$  of dentin is present and the above UV dose is used, no more than 3  $\text{mJ}/\text{cm}^2$  of UV light will pass to the surrounding tissues. This value is within the safety limits defined by the American Conference of Governmental Industrial Hygienists<sup>28</sup> and adopted by the Navy Environmental Health Center and the National Institute of Occupational Safety and Health.

It may be important not to use UV light in canals with insufficient remaining dentin thickness ( $<100 \mu\text{m}$ ). However, this will hardly be a problem when modern nickel titanium rotary files are used.

Illumination through the apical foramen and potential perforations should also be avoided. The former may easily be handled by blocking the light passage through the tip of the diffuser by applying an opaque layer on its tip. For the latter, an electronic apex locator should be used to verify that no perforation exists in a given root canal.

This preliminary report indicates that application of UV light after NaOCl disinfection of root canals may be a feasible, immediate, and effective way to achieve thorough disinfection. Nevertheless, further efficacy and safety studies will be required before this concept may turn into a clinical reality.

## CONCLUSIONS

1. Sodium hypochlorite alone failed to thoroughly disinfect *E. faecalis*-infected root canals in vitro, and viable bacteria were found in 53% of them.
2. Application of 254-nm UV light to the canal walls as a supplementary agent after NaOCl application rendered 96% of the root canals free of viable bacteria.
3. Obturated root canals maintained this status for 14 days.

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