Effects of Chemomechanical Preparation With 2.5% Sodium Hypochlorite and Intracanal Medication With Calcium Hydroxide on Cultivable Bacteria in Infected Root Canals

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Abstract

This clinical study was conducted to assess the bacterial reduction after chemomechanical preparation with 2.5% NaOCl as an irrigant and the additive antibacterial effect of intracanal dressing with calcium hydroxide. According to stringent inclusion criteria, 11 teeth with primary intraradicular infections and chronic apical periodontitis were selected and monitored in the study. Bacterial samples were taken at the baseline (before treatment) (S1), after chemomechanical preparation with 2.5% NaOCl as an irrigant (S2), and after a 7-day dressing with a calcium hydroxide paste in glycerin (S3). Cultivable bacteria recovered from infected root canals at the 3 stages were counted and identified by means of 16S rRNA gene sequencing analysis. At S1, all canals were positive for bacteria, with the mean number of 2.8 taxa per canal (range, 1–6). At S2, 5 cases (45.5%) still harbored cultivable bacteria, with 1 or 2 species per canal. At S3, bacteria were cultured from 2 cases (18.2%), with 1 species per positive case. There was no indication that any specific bacterial taxon was more resistant to treatment. A significant reduction in bacterial counts was observed between S1 and S2, and S1 and S3. However, no statistically significant difference was observed for comparisons involving S2 and S3 samples with regard to the number of cases yielding negative cultures ($P = .18$) or quantitative bacterial reduction ($P = .19$). It was concluded that the whole antibacterial protocol used in this study significantly reduced the number of bacteria in the canal and rendered most canals free of cultivable bacteria. *(J Endod 2007;33:800–805)*

Key Words

16S rRNA gene sequencing, antimicrobial treatment, apical periodontitis, endodontic microbiology

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Primary intraradicular infections are largely nonspecific and predominantly anaerobic, with no single species being considered as the main pathogen. These infections display a large interindividual variability, ie, each individual exhibits a unique endodontic microbiota in terms of composition and species dominance (1, 2). Consequently, the endodontic treatment should rely on a broad-spectrum antimicrobial strategy to deal with such infections.

Because of the privileged anatomic localization, bacteria entrenched in the root canal system are beyond the reaches of the host defenses. Therefore, endodontic infections can only be treated by means of professional intervention with both chemical and mechanical procedures. The main steps of endodontic treatment involved with control of the infection are represented by the chemomechanical preparation and the interappointment medication. In this regard, the chemomechanical preparation is of paramount importance for root canal disinfection, because instruments and irrigants act primarily on the main canal, which is the most voluminous area of the system and consequently harbors the largest number of bacterial cells. Bacterial elimination from the root canal is carried out by means of the mechanical action of instruments and the flow and backflow of the irrigant solution as well as the antibacterial effects of irrigants. Several irrigants have been proposed over the years, but sodium hypochlorite (NaOCl) remains the most widely used one. However, studies have revealed that instrumentation and irrigation with NaOCl per se do not suffice to predictably render root canals free of cultivable bacteria (3–7). About 40%–60% of the canals still contain cultivable bacteria after chemomechanical preparation with different NaOCl concentrations (3–7). This is because endodontic instruments have a design and rigidity that allow them to act only in the main canal, and NaOCl remains in the canal for a short period of time, which might be insufficient to reach other areas of the root canal system, including irregularities, lateral canals, isthmuses, apical deltas, and dentinal tubules.

The use of an interappointment antibacterial medication has been recommended mostly to eliminate bacteria not affected by the chemomechanical preparation. Calcium hydroxide is arguably the most commonly used intracanal medication, and several clinical studies have reported on its antibacterial activity (3, 5, 7–15). Nevertheless, its effectiveness in significantly increasing the number of culture-negative canals after chemomechanical procedures has been somewhat inconsistent (5, 11, 13).

Most clinical studies investigating the antibacterial effects of intracanal procedures have provided only quantitative data with no species identification. The knowledge of the species that can be resistant to treatment assumes special relevance to the establishment of therapeutic strategies effective in eliminating these species. The few studies that have identified bacteria persisting treatment have made use of phenotypic characterization of the isolates. However, it is well-known that a precise bacterial identification cannot always be achieved with phenotype-based methods. The 16S rRNA gene sequencing method has emerged as a valuble tool for the identification of bacterial isolates, and unlike phenotype-based tests, this molecular approach can provide unambiguous data for identification of isolates that are unreactive in biochemical tests, species with atypical phenotypes, rare isolates, or poorly described bacteria (16). Furthermore, the technique can lead to recognition of novel species and previously uncultivated bacteria (16). Identification of cultivable bacteria on the basis of the 16S rRNA gene sequencing has been recently adopted by many clinical microbiology laboratories to identify rarely

isolated or previously uncharacterized species as well as isolates that are difficult to identify by conventional methods (17).

The purpose of the present study was to assess the effects of chemomechanical preparation with 2.5% NaOCl as an irrigant and intracanal dressing with calcium hydroxide on cultivable bacteria from primary intraradicular infections of teeth with apical periodontitis. Cultivable bacteria recovered from infected root canals at 3 treatment stages were identified by means of 16S rRNA gene sequencing analysis.

Material and Methods

Clinical Material

Patients presenting to the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil for evaluation and treatment of apical periodontitis were considered for this study. Twelve single-rooted teeth (4 maxillary central incisors, 4 maxillary lateral incisors, 3 mandibular incisors, and 1 mandibular premolar) from 10 patients (8 women and 2 men; aged 24–54 years; mean, 38.7 years) were selected for this study on the basis of stringent criteria. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of chronic apical periodontitis lesions were included in this study. The size of the apical periodontitis lesions ranged from 2 × 3 mm to 12 × 15 mm. Teeth from patients who received antibiotic therapy within the previous 3 months, teeth with gross carious lesions, teeth with fractures of the root or crown, teeth that had received previous endodontic treatment, and cases showing periodontal pockets greater than 4 mm deep were excluded from the study. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Endodontic Treatment and Sampling Procedures

Rubber dam and an aseptic technique were used throughout the endodontic treatment. Before isolation with rubber dam, each tooth had supragingival plaque removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. After rubber dam application, dental floss was securely tied around the neck of the tooth. The operative field, including the tooth, clamp, and surroundings, were cleaned with 3% hydrogen peroxide until no further bubbling of the peroxide occurred. All surfaces were then disinfected by vigorous swabbing with a 2.5% NaOCl solution. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was then cleaned and disinfected once again the same way as above. NaOCl was neutralized with 5% sodium thiosulfate, and then sterility control samples were taken from the tooth surface with sterile paper points. For inclusion of the tooth in the study, these control samples had to be uniformly negative.

The first root canal sample (S1) was taken as follows. Three sterile paper points were consecutively placed in the canal to a level approximately 1 mm short of the tooth apex, on the basis of diagnostic radiographs, and used to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute. Paper points were then transferred aseptically to tubes containing 500 μL of reduced transport fluid (RTF).

Chemomechanical instrumentation was completed at the same appointment in all cases. The alternated rotation motion (ARM) technique was used to prepare all canals (18). Briefly, the coronal two thirds of the root canals were enlarged with Gates-Glidden (Moyco Union Broach, York, PA) burs. Working length was established 1 mm short of the root apex, and the patency length coincided with the radiographic root edge. Apical preparation was completed to the working length with hand nickel-titanium files (Nitiflex; Dentsply-Maillefer, Ballaigues, Switzerland), always using a back-and-forth ARM. Master apical files ranged from #50 to #55, depending on both root anatomy and initial diameter of the root canal. Apical patency was confirmed with a small file (#15 or #20 NitiFlex) throughout the procedures after each larger file size. Preparation was completed by using step-back of 1-mm increments. NaOCl (2.5%) was used as an irrigant during the preparation. Two milliliters of this solution was used to rinse the canals after each instrument. Irrigant was delivered in the canals by means of a 5-mL disposable syringe with a 23-gauge needle.

Each canal was dried by using sterile paper points and then flushed with 5 mL of 5% sodium thiosulfate to inactivate the NaOCl. Subsequently, the root canal walls were gently filed, and a postinstrumentation sample (S2) was taken from the canal as described above.

To remove the smear layer, 17% ethylenediaminetetraacetic acid was left in the canal for 3 minutes followed by irrigation with 5 mL of 2.5% NaOCl. The canal was dried with paper points and dressed with a mix of calcium hydroxide in glycerin, which was placed in the canals by means of lentulo spiral fillers. The calcium hydroxide slurry was packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide in the canal. The access cavities were filled with at least 4-mm thickness of a temporary cement (Coltosol; Coltène/Whaledent Inc, Cuyahoga Falls, OH).

The second appointment was scheduled for 1 week thereafter. At this time, the tooth was isolated with a rubber dam, the operative field was disinfected, and the NaOCl was neutralized, as outlined earlier. A sterility control sample of the operating field was obtained. The temporary filling was removed, and the calcium hydroxide paste was rinsed out of the canal by using sterile saline solution and the master apical file. The root canal walls were filed lightly to remove loose calcium hydroxide remnants, and a postmedication sample (S3) was taken from the canals. Subsequently, the canals were filled with gutta-percha and Sealer 26 (Dentsply, Petrópolis, RJ, Brazil) by using cold lateral compaction. The tooth was sealed temporarily with glass ionomer cement, and a permanent restoration was planned. All clinical procedures were conducted by one experienced endodontist (T.G.P.).

Microbiologic Analysis

Samples were transported to the laboratory within 15 minutes for microbiologic processing. Samples in RTF vials were dispersed with a vortex for 30 seconds, and 10-fold serial dilutions to 10^{-3} (for S1 samples) or 10^{-2} (for S2 and S3 samples) were made in prereduced anaerobically sterilized buffered salt solution. Aliquots of 100 μL from the undiluted suspension and the highest dilution were each spread onto Brucella agar plates (BBL Microbiology Systems, Cockeysville, MD) supplemented with 5% defibrinated sheep blood, hemin (5 mg/L), and menadione (1 mg/L), and Mitis-salivarius agar plates (Difco, Detroit, MI). Plates were incubated anaerobically within anaerobic jars (GasPak system; BBL Microbiology Systems) at 37°C for 14 days. After incubation, the total colony-forming units (CFUs) were counted, and actual counts were calculated on the basis of the known dilution factors. One or 2 colonies of each different colony type were isolated, and each one was individually placed in cryovials containing TE buffer (10 mmol/L Tris-HCl, 1 mmol/L ethylenediaminetetraacetic acid, pH 8). Cryovials were then stored at –20°C until further bacterial identification by 16S rRNA gene sequencing.

16S rRNA Gene Identification

Genomic DNA was extracted from each colony by heating the suspension for 10 minutes at 97°C with a thermocycler. The vials were then stored for 5 minutes on ice and centrifuged, and 5-μL aliquots of the supernatant were further used as template in the polymerase chain reaction (PCR) assay.

TABLE 1. Bacterial Counts and Percent Reduction Determined for Root Canal Samples of 11 Teeth with Apical Periodontitis Lesions

Case	Initial Samples (S1)	Postinstrumentation Samples (S2)	Postmedication Samples (S3)
1TT	3×10^5	0 (100%)	0 (100%)
2TT	7.65×10^5	0 (100%)	0 (100%)
4TT	5.49×10^5	2.77×10^5 (49.54%)	3×10^2 (99.95%)
5TT	2.39×10^4	(100%)	0 (100%)
6TT	2×10^8	1×10^7 (95%)	0 (100%)
7TT	2×10^5	5.1×10^3 (97.45%)	0 (100%)
8TT	1.08×10^7	9.2×10^3 (99.91%)	3.76×10^5 (96.52%)
9TT	3.24×10^4	0 (100%)	0 (100%)
10TT	6×10^6	2.4×10^3 (99.96%)	0 (100%)
11TT	8.8×10^3	0 (100%)	0 (100%)
12TT	4×10^3	0 (100%)	0 (100%)
Median	3×10^5	0	0

Samples were taken before treatment, after instrumentation with 2.5% NaOCl as an irrigant, and after 7 days of calcium hydroxide intracanal medication.

PCR amplification of 16S rRNA genes was used for bacterial identification. The pair of universal 16S rRNA gene primers used were 5′-GAT TAG ATA CCC TGG TAG TCC AC-3′ and 5′-CCC GGG AAC GTA TTC ACC G-3′, corresponding to base positions 786–808 and 1369–1387, respectively, and spanning the variable regions V5–V8 of the *Escherichia coli* 16S rRNA gene. PCR amplification was performed in a reaction volume of 50 μL, consisting of 0.8 μmol/L concentration of each primer, 5 μL of 10 × PCR buffer, 2 mmol/L $MgCl_2$, 1.25 U of *Tth* DNA polymerase, 0.2 mmol/L concentration of each deoxyribonucleoside triphosphate (all reagents from Biotools, Madrid, Spain). Cycling parameters included an initial denaturation step at 95° C for 2 minutes, followed by 36 cycles of a denaturation step at 95°C for 30 seconds, a primer annealing step at 60°C for 1 minute, an extension step at 72°C for 1 minute, and a final step of 72°C for 2 minutes. The results of PCR amplification were examined by electrophoresis in 1.5% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength ultraviolet light.

PCR products were purified with a PCR purification system (Wizard PCR Preps; Promega, Madison, WI) and then sequenced directly on the ABI 377 automated DNA sequencer with dye terminator chemistry (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). Sequence data and electropherograms were inspected and corrected when obvious sequencing software errors were observed. Sequences generated were compared with the GenBank database to identify the closest relatives by using the BLAST algorithm (19). A ≥99% identity in 16S rRNA gene sequence was the criterion used to identify an isolate to the species level. A 97%–99% identity in 16S rRNA gene sequence was the criterion used to identify an isolate at the genus level, whereas <97% identity in 16S rRNA gene sequence was the criterion used to define a potentially new bacterial species (17).

Statistical Analysis

Effectiveness of each treatment step in rendering root canals free of cultivable bacteria was recorded as percentage of cases yielding negative cultures. In this regard, the Fisher exact test was used to compare S2 and S3 samples. Percent reduction in the number of CFUs after each treatment step was calculated on the basis of quantitative data obtained from samples S1, S2, and S3. Quantitative data were statistically analyzed for differences with the Mann-Whitney test comparing pairs of groups. Significance level was always set at 5% ($P < .05$).

Results

Of the 12 teeth sampled, one showed bacterial growth for the sterility control of the working field and was excluded from the study. Bacteria were found in all initial samples (S1) from the other 11 root canals. The median value of the number of CFUs in the initial samples was 3×10^5, ranging from 4×10^3 to 2×10^8.

After chemomechanical preparation with 2.5% NaOCl as an irrigant, 6 of the 11 canals (54.5%) showed negative culture results. The median number of CFUs in postinstrumentation samples was 0, ranging from 0–1×10^7. When compared with initial samples, chemomechanical preparation promoted reduction in the number of bacteria ranging from 49.54%–100%.

After 7 days of dressing with calcium hydroxide paste, 9 of the 11 canals (81.8%) yielded no cultivable bacteria. The median number of CFUs in postmedication samples was 0, ranging from 0–3.76×10^5. Percent reduction in the bacterial population after dressing with calcium hydroxide ranged from 96.52%–100%.

Analysis of the quantitative data revealed that the number of CFUs in S2 and S3 was significantly reduced in comparison with S1 ($P = .004$ and $P < .001$, respectively). No significant difference was observed for comparisons involving S2 and S3 samples with regard to the number of cases yielding negative cultures ($P = .18$, Fisher exact test) or quantitative bacterial reduction ($P = .19$, Mann-Whitney test). Quantitative data and percent reductions are depicted in Table 1 and illustrated in Fig. 1.

Twenty-eight isolates belonging to 20 bacterial taxa were identified in initial samples. Three other isolates were not identified because of sequences with low-scoring homologies (<97% similarity) to sequences deposited in the GenBank. The mean number of bacterial taxa per canal was 2.8, ranging from 1–6. The most prevalent taxa were *Streptococcus* species, *Fusobacterium nucleatum*, and *Actinomyces israelii* (Table 2).

Seven isolates belonging to 7 distinct taxa were recovered from S2 samples (mean, 1.4 taxa per case). Of the 5 cases showing positive results, 3 cases harbored 1 taxon each (*Streptococcus gordonii*, a *Flavobacterium* species, or *Staphylococcus epidermidis*), whereas the other 2 cases harbored 2 taxa each (*Streptococcus mitis* biovar 2 and *Staphylococcus aureus*, or *Neisseria sicca* and *Streptococcus oralis/mitis/sanguinis*). All these bacteria were also found in S1, but none was isolated again from the canals in S3.

Two taxa were isolated from the only 2 cases that sampled bacteria after dressing with calcium hydroxide. *Fusobacterium nucleatum* was found in 1 case (4TT) and was also detected in S1 but not in S2. *Lactococcus garvieae* was found in the other case (8TT), although it was present neither in S1 nor in S2. There was no indication that any specific bacterial taxon was more resistant to treatment.

Discussion

In the present study, anaerobic culture procedures followed by identification of the isolates by 16S rRNA gene sequencing were used to investigate the effectiveness of a treatment protocol in eliminating bacteria from the root canal. Although several different species were iden-

tified, it is important to remember that this is not a comprehensive analysis of the microbiota. It has been well-established that approximately 40%–55% of endodontic bacteria have not yet been cultivated by standard culture techniques (1, 20). Therefore, presence of many taxa might have been overlooked in our samples. As a consequence, it is entirely possible that the number of taxa per positive case might well have been higher, and that cases showing negative cultures harbored indeed fastidious and/or as yet uncultivated bacteria. Even aware of the method's limitations, culture was used because it is one of the most reliable methods to detect viable bacteria, particularly when samples are taken immediately after antibacterial treatment in which viability might not be ascertained by most culture-independent methods (16). In addition, studies with culture have shown a correlation between negative cultures and a favorable treatment outcome (6, 21). 16S rRNA gene sequencing analysis was used to achieve a more precise identification of cultivable bacteria from infected root canals.

All cases cultured bacteria at initial samples (S1), confirming the strong correlation between bacteria and apical periodontitis. The initial number of bacteria in the infected root canals varied between 10^3 and 10^8. These figures are comparable to previous studies (11, 22). Substantial bacterial reduction was observed after chemomechanical preparation, which also parallels other findings from the literature (4, 5, 18). Most cases instrumented and irrigated with 2.5% NaOCl had the number of bacteria reduced 10^2-fold to 10^5-fold. Except for 1 case (4TT), percent reduction of the bacterial counts was always more than 95%. These findings confirm the important role played by chemomechanical preparation in reducing the bacterial population in infected canals.

Although bacterial reduction after chemomechanical preparation was significant in quantitative terms, 45.5% of the infected root canals were still positive for the presence of bacteria after instrumentation and irrigation with 2.5% NaOCl. This figure is within the range reported by other studies (3–7). Although no single species was found in more than 1 canal, streptococci as a group were found in 3 of the canals that sampled bacteria in S2. Other studies have reported streptococci as one of the most commonly found species in postinstrumentation samples positive for bacteria (4, 23, 24). However, none of these species resisted a 7-day intracanal medication with calcium hydroxide in the present study.

After interappointment dressings with calcium hydroxide for 1 week, only 2 cases (18.2%) still sampled bacteria, with 1 taxon per case. *F. nucleatum* was cultivated from one of these cases after being present in S1, which indicates persistence after treatment. The fact that this species was not isolated from S2 might be related to failure in the sampling approach or presence in numbers below the sensitivity of the culturing method. *F. nucleatum* has not been generally considered a bacterial species resistant to treatment, but findings from some studies might argue otherwise. *F. nucleatum* has been found in samples taken after chemomechanical preparation with NaOCl as an irrigant (4, 6) and in root canal–treated teeth with persistent disease (25, 26), although in a few cases. An in vitro study demonstrated that *F. nucleatum* colonizing dentin specimens might be resistant to calcium hydroxide medication (27), which might help explain the present detection of this species in the postmedication sample.

The other case yielding positive culture at S3 showed an increase in the number of CFUs when compared with S2. A bacterial species (*L. garvieae*) not found in S1 and S2 samples was identified. Although this bacterial species might well have been present in S1 and S2 in levels below the sensitivity of culture, occurrence of this species in the canal only in S3 might be better explained by sample contamination from the tooth crown or canal contamination between appointments as a result of leakage through the temporary restorative material. The negative sterility control sample points to the latter possibility, even though the temporary restoration was apparently intact. *L. garvieae*, which is synonymous with *Enterococcus seriolicida*, is an important pathogen in aquaculture but rarely causes disease in humans, although cases of human endocarditis with involvement of *L. garvieae* have been recently reported (28, 29). Also, patients with *L garvieae* infection are usually associated with gastrointestinal disorders and have a history of consuming raw fish (30), which might have contributed to the oral presence and canal contamination by this species.

It has been demonstrated that some microbial species found in infected root canals, such as *Enterococcus faecalis* and *Candida albicans*, can be resistant to calcium hydroxide (8, 31). However, these 2 species were not found in any of the samples analyzed herein. *E. faecalis* and *C albicans* have been reported to be significantly more prevalent in root canal–treated teeth with persistent apical periodontitis lesions (25, 26). During retreatment of these cases, the use of calcium hydroxide with an inert vehicle, such as distilled water, saline, or glycerin, should be rethought. Combinations of calcium hydroxide with other disinfectants, such as chlorhexidine or camphorated paramonochlorophenol, present a broader spectrum of antimicrobial activity (32) and thereby have the potential to be more effective in retreatment cases.

No significant difference was found for absolute bacterial counts between S2 and S3. Although the number of cases showing negative cultures increased from 54.5% after chemomechanical preparation to

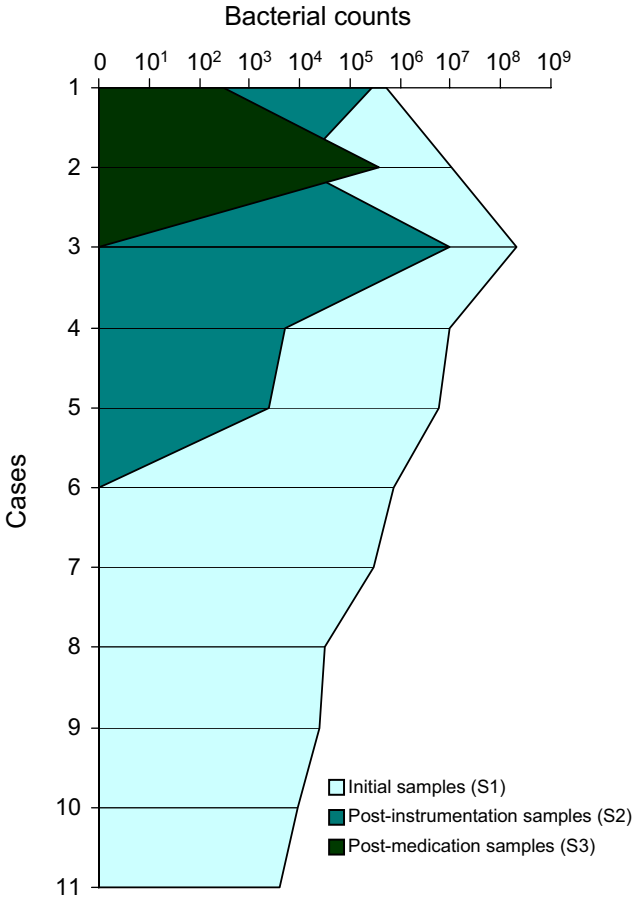

Figure 1. Bacterial reduction per case after chemomechanical preparation with 2.5% NaOCl as an irrigant and a 7-day intracanal dressing with calcium hydroxide/glycerin paste. Cases are ordered according to the bacterial counts after treatment steps.

TABLE 2. Cultivable Bacterial Taxa Detected during Treatment of 11 Infected Root Canals Associated with Apical Periodontitis Lesions

Bacteria Only in Initial Samples (S1)*	Persisting Bacteria	
	Postinstrumentation (S2)†	Postmedication (S3)
Actinomyces israelii (2)	*Streptococcus mitis* biovar 2 (1)	*Fusobacterium nucleatum* (1)‡
Streptococcus anginosus (2)	*Streptococcus gordonii* (1)	*Lactococcus garvieae* (1)§
Streptococcus mitis biovar 2 (2)	*Streptococcus oralis/mitis/sanguinis* (1)	
Streptococcus parasanguinis (2)	*Neisseria sicca* (1)	
Fusobacterium nucleatum (1)	*Flavobacterium* sp (1)	
Prevotella marshii (1)	*Staphylococcus aureus* (1)	
Campylobacter rectus (1)	*Staphylococcus epidermidis* (1)	
Actinomyces oral clone GU009 (1)		
Actinomyces sp (1)		
Propionibacterium acnes (1)		
Streptococcus gordonii (1)		
Streptococcus sp oral strain T4-E3 (1)		
Streptococcus oralis/mitis/sanguinis (1)		
Rothia dentocariosa (1)		
Rothia mucilaginosa (1)		
Delftia tsuruhatensis (1)		
Unidentified (3)		

Data are based on 16S rRNA gene sequencing identification of isolates.

*Bacteria eliminated after treatment and not present in S2 or S3.

†Bacteria found in both S1 and S2.

‡Isolate found in S1 but not in S2.

§Isolate found neither in S1 nor in S2.

81.8% after calcium hydroxide dressing, the difference was not significant either. At first glance, it is reasonable to surmise that by increasing the sample size, the statistical significance could easily become apparent. Even so, these results are in agreement with many other studies that showed that although the number of negative cultures increased after calcium hydroxide application, some canals still had cultivable bacteria (3, 5) (Table 3). Although calcium hydroxide can rapidly kill most endodontic bacteria when in direct contact, these effects might not be so pronounced in the root canal environment, in which such a direct contact cannot always be achieved, or given the low solubility of calcium hydroxide, the concentration of hydroxyl ions might not reach levels sufficiently high to kill bacteria in biofilms on untouched canal walls, dentinal tubules, irregularities, and other anatomic variations (32).

It is salient to point out that attainment of a negative culture does not imply that the canal is free of bacteria. In these culture-negative cases, bacteria might be in regions inaccessible to sampling procedures (which are usually limited to the main canal), they might be in the canal in levels below the sensitivity of the culture method, or they can be unable to grow under artificial laboratory conditions (as yet uncultivated bacteria). These 3 situations have already been demonstrated by microscopic (33) and molecular biology studies (24, 34, 35). However, studies have demonstrated that the chance for a better prognosis of the endodontic treatment significantly increases when the root canal does not culture bacteria at the root canal–filling stage (6, 21). On the basis of this premise, it is reasonable to assume that an intracanal medication with calcium hydroxide might be necessary to get a high incidence of negative cultures before filling and then improve the outcome of the endodontic treatment.

In conclusion, a statistically significant reduction in the number of cultivable bacteria from infected root canals was obtained following the

TABLE 3. Data from Different Studies Showing the Incidence of Positive Cultures in Root Canals Dressed with Calcium Hydroxide for Different Time Intervals

Study	Irrigation	Medication	Time of Medication	Positive Cultures*
Byström et al, 1985 (8)	*0.5% or 5% NaOCl*	Calcium hydroxide	30 days	0/35 (0%)† 1/35 (3%)‡
Reit and Dahlén, 1988 (9)	0.5% NaOCl	Calcium hydroxide	14 days	8/32 (25%)† 9/32 (28.1%)‡
Orstavik et al, 1991 (10)	Saline	Calcium hydroxide	7 days	8/22 (36.4%)†
Sjögren et al, 1991 (11)	0.5% NaOCl	Calcium hydroxide	7 days	0/18 (0%)† 0/18 (0%)‡
Shuping et al, 2000 (5)	1.25% NaOCl	Calcium hydroxide	7–203 days	3/40 (7.5%)†
Lana et al, 2001 (12)	2.5% NaOCl	Calcium hydroxide	7 days	4/27 (14.8%)† 7/27 (25.9%)‡
Peters et al, 2002 (13)	2% NaOCl	Calcium hydroxide	28 days	15/21 (71.4%)†
Kvist et al, 2004 (7)	0.5% NaOCl	Calcium hydroxide	7 days	16/43 (37.2%)†
McGurkin-Smith et al, 2005 (3)	5.25% NaOCl	Calcium hydroxide	7–110 days	4/24 (16.7%)†
Zerella et al, 2005 (14)§	1% NaOCl	Calcium hydroxide	7–10 days	10/20 (50%)†
Chu et al, 2006 (15)	0.5% NaOCl	Calcium hydroxide	7 days	11/35 (31.4%)†
Siqueira et al (this study)	2.5% NaOCl	Calcium hydroxide	7 days	2/11 (18.2%)†

*Number of cases positive for bacteria in post-treatment samples/Number of cases positive for bacteria in initial samples.

†Samples taken at the same visit as when the dressing was removed.

‡Samples taken some days after the dressing was removed and the canal was left empty.

§Retreatment cases.

protocol used. No cultivable bacteria were isolated from 54.5% of the canals after instrumentation with hand NiTi files and irrigation with 2.5% NaOCl. The number of cases showing negative cultures further increased to 81.8% after smear layer removal and a 7-day intracanal medication with calcium hydroxide in an inert vehicle (glycerin). Although this difference did not reach statistical significance, it appears that an increase of almost 30% in the number of culture-negative cases would be reasonable to justify the use of calcium hydroxide medication. Even so, the fact that some cases still harbored bacteria after the whole treatment protocol points to the need to develop more effective strategies to predictably render canals bacteria free.

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