Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis

C. Sathorn, P. Parashos & H. Messer
Endodontic Unit, School of Dental Science, The University of Melbourne, Melbourne, Vic. Australia

Abstract

Aim To determine to what extent does calcium hydroxide intracanal medication eliminate bacteria from human root canals, compared with the same canals before medication, as measured by the number of positive cultures, in patients undergoing root canal treatment for apical periodontitis (teeth with an infected root canal system).

Methodology CENTRAL, MEDLINE and EMBASE databases were searched. Reference lists from identified articles were scanned. A forward search was undertaken on the authors of the identified articles. Papers that had cited these articles were also identified through the Science Citation Index to identify potentially relevant subsequent primary research.

Review methods The included studies were pre-/post-test clinical trials comparing the number of positive bacterial cultures from treated canals. Data in those studies were independently extracted. Risk differences of included studies were combined using the generic inverse variance and random effect method.

Results Eight studies were identified and included in the review, covering 257 cases. Sample size varied from 18 to 60 cases; six studies demonstrated a statistically significant difference between pre- and post-medicated canals, whilst two did not. There was considerable heterogeneity among studies. Pooled risk difference was 21%; 95% CI: −47% to 6%. The difference between pre- and post-medication was not statistically significant ($P = 0.12$).

Conclusions Calcium hydroxide has limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques.

Keywords: canal disinfection, infection control, microbiology.

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Introduction
Calcium hydroxide has been used in dentistry for almost a century (Siqueira & Lopes 1999). Its use in root canal treatment as an intracanal medication has been associated with periradicular healing (Sjögren et al. 1990) and few adverse reactions (De Moor & De Witte 2002). Its use in root canal treatment was promoted by a series of papers (Byström & Sundqvist 1985, Byström et al. 1985) documenting the antibacterial efficacy of calcium hydroxide in human root canals. Subsequent studies substantiated these reports (Ørstavik et al. 1991, Sjögren et al. 1991), and the routine use of calcium hydroxide as an inter-appointment intracanal medicament became widespread.

Apical periodontitis is caused by bacteria within the canal space (Kakehashi et al. 1965, Möller et al. 1981). The treatment of apical periodontitis should, therefore, aim at bacterial eradication. Because cleaning and shaping procedures alone do not reliably
eliminate bacteria (Byström & Sundqvist 1981, Dalton et al. 1998), it seems logical to medicate canals with an antibacterial agent after canal preparation. Recently, the ability of calcium hydroxide medication to eradicate completely bacterial species from the root canal has been questioned. For example, ex vivo studies have shown that dentine can inactivate the antibacterial activity of calcium hydroxide (Haapasalo et al. 2000, Portenier et al. 2001) and one clinical study (Peters et al. 2002) has shown that the number of bacterial positive canals increased after calcium hydroxide medication. Other studies have indicated that calcium hydroxide could not reliably eliminate bacteria or that cultures changed from negative to positive after calcium hydroxide medication (Peters et al. 2002, Reit et al. 1999, Waltimo et al. 2005).

When different studies report inconsistent results, a systematic review and meta-analysis technique can clarify conflicting research data and the current state of knowledge regarding specific issues. A systematic review is a method of systematically identifying relevant research, appraising its quality and synthesizing the results (Glasziou 2001). Such a review of the antibacterial efficacy of calcium hydroxide has been conducted (Law & Messer 2004); however, since its publication, there have been three further clinical studies published that provide more complete information on culture results. In addition, the previous review did not perform a meta-analysis, on the grounds that the level of evidence was too low for such an analysis to be of value.

The ideal clinical question to be answered in this systematic review can be framed in terms of a PICO question [problem (P), intervention (I), comparison (C) and outcome (O)] as follows: in patients undergoing endodontic treatment for apical periodontitis, does an intracanal medicament, compared with no intracanal medicament, result in elimination of bacteria from the root canal system, as measured by a negative culture? Only one of the eight studies evaluated included a control group (no intracanal medication), on the premise that bacteria multiply rapidly when canals are left empty between appointments (Byström & Sundqvist 1981. Byström et al. 1985, Yoshida et al. 1995). Thus, it was necessary to restate the question to compare the bacterial status of the same canals before and after medication: in patients undergoing root canal treatment for apical periodontitis (teeth with an infected root canal system), to what extent does calcium hydroxide medication eliminate residual bacteria from human root canals, compared with the same canals before medication, as measured by the number of positive cultures?

Most clinical studies sample canals for bacterial culture at three stages: (i) after initial access, to confirm that the canal is infected at the time of treatment (designated S1); (ii) after the cleaning and shaping procedure is complete, immediately before canal medication (S2); (iii) when the canal is re-accessed 1–4 weeks later, after the medication has been removed (S3). Teeth with apical periodontitis (as manifested by a periapical radiolucency) routinely have an infected root canal system; hence S1 is positive in essentially 100% of cases (Sundqvist 1976). Canal debridement (cleaning and shaping) results in an extensive reduction in bacterial count (99–99.9%) and in most studies a substantial proportion of negative cultures has been reported at S2 (0–86%, pooled mean 38% in the review by Law & Messer 2004). It has been shown that calcium hydroxide could interfere with the validity of microbiological sampling (Reit et al. 1999). To take this issue into account, ideally, teeth with negative cultures at S2 should be tracked separately from those with positive cultures in assessing the effectiveness of calcium hydroxide as measured at S3. This information was generally lacking in the studies reviewed by Law & Messer (2004). For this meta-analysis, the data were obtained either by personal contact with the authors or derived from the published data where available.

Materials and methods

Literature search

An exhaustive search was undertaken to identify all clinical studies that compared the microbiological status of pre- and post-mediated human root canals. The MEDLINE database was searched via the EviDents search engine (http://medinformatics.uthscsa.edu/Evi-Dents/, last accessed 20th December 2005) using ‘calcium hydroxide’ and ‘bacteria’ as keywords, which automatically created a complex search strategy (Table 1). The same search strategy was also applied using CENTRAL and EMBASE databases. This complex search strategy was similar to the one recommended by the Cochrane Collaboration as outlined in the Cochrane Reviewers’ Handbook (Alderson et al. 2004). The search of the MEDLINE database included all years from 1966 to December 2005. A similar
search was undertaken on EMBASE (1988–2005). In addition a thorough search of six thesis databases (The Networked Digital Library of Theses and Dissertations, The Proquest Digital Dissertations, OAIster, Index to theses, The Australian Digital Thesis program and Dissertation.com) and one conference report database (BIOSIS Previews®) was undertaken in an attempt to retrieve unpublished data. No language restriction was applied to the search. One hundred and fifty-four studies were subjected to the preliminary analysis. Titles and abstracts, where available, were scanned and the relevance of each study to the antibacterial efficacy of calcium hydroxide was determined. Where information from the title and abstract was not adequate in determining the paper’s relevance, the paper was automatically included in subsequent analysis. One hundred and forty-three studies were excluded from the list, and the 11 remaining articles were subjected to stricter exclusion criteria.

**Inclusion and exclusion**

The full texts of the remaining papers were then obtained and reviewed, and the inclusion criteria (Table 2) were applied. Reference lists from identified articles were scanned to identify other potentially relevant preceding articles (a backward search) (three more articles were identified: Reit & Dahlen 1988, Safavi et al. 1985, Sundqvist et al. 1998). A forward search was undertaken on the authors of the identified articles. Papers that had cited these articles were also identified through the Science Citation Index (http://www.isinet.com), to identify potentially relevant subsequent primary research (Glasziou 2001) (two more articles were identified: McGurkin-Smith et al. 2005, Waltimo et al. 2005).

**Data extraction**

A systematic data extraction sheet was constructed. All aspects of treatment that could potentially affect the study outcomes were identified and included in the data sheet. The data in all included studies were extracted in the same fashion. Authors of three studies were contacted to acquire additional information not available in the published article namely, the number of teeth with negative cultures at S2 that were positive at S3, i.e. culture reversal.

**Table 2** Inclusion and exclusion criteria used in the analysis

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>1. Subjects had a noncontributory medical history</td>
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<tr>
<td>2. Subjects presented with mature teeth and radiographic evidence of periapical bone loss (as an indication of preoperative canal infection)</td>
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<tr>
<td>3. All selected root canals had not received any endodontic treatment previously</td>
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<td>4. Subjects underwent nonsurgical root canal treatment during the study</td>
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<td>5. Teeth were dressed with calcium hydroxide sealed in the canals</td>
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<tr>
<td>6. Microbiological sampling was undertaken during the course of treatment, before canal preparation (S1), after canal preparation (S2) and after canal medication (S3)</td>
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<td>7. Aerobic and anaerobic culturing techniques were performed on all samples</td>
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<td>8. Treatment outcome was stated in terms of positive and negative canal cultures</td>
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</tbody>
</table>

<table>
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<tr>
<th>Exclusion criteria</th>
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</thead>
<tbody>
<tr>
<td>1. Inclusion of test teeth without infected necrotic root canal systems and/or radiographic evidence of periapical bone loss (hence no preoperative canal infection)</td>
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<tr>
<td>2. Study carried out on failed, endodontically treated teeth (retreatment cases)</td>
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<tr>
<td>3. No post-instrumentation sample (S2)</td>
</tr>
<tr>
<td>4. Post-medication sample (S3) not taken immediately after removal of the test medicament</td>
</tr>
<tr>
<td>5. Use of multiple antibacterial medicaments in succession, in the same canal</td>
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<tr>
<td>6. Repeated cleaning and irrigation procedures in multiple appointments</td>
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</table>
Meta-analysis

Between-study heterogeneity was assessed using the standard chi-squared test or Q-statistic. The principal measure of treatment effect (antibacterial efficacy) was risk difference, which is normally defined as the risk in the experimental group minus risk in the control group. For the purpose of this study it is given as the difference in the proportion of bacterial positive cultures between pre- and post-medication (S2 versus S3). Risk difference is a measure of the impact of the treatment on the number of events (the number of positive cultures), as it takes into account the prevalence of the event, i.e. how common it is (Sutton 2000). Risk differences of included studies were combined as generic inverse variance data type (RevMan Version 4.2.7, The Cochrane Collaboration’s Information Management System, http://www.cc-ims.net, last accessed 19 December 2005), taking into account the separate tracking of positive and negative cultures at S2. The random effects model for combining study estimates was used and an overall estimate was produced (Sutton 2000). The level of statistical significance was set at 0.05.

Results

Included and excluded studies

Eight studies met the inclusion criteria (Table 2): Kvist et al. (2004), McGurkin-Smith et al. (2005), Ørstavik et al. (1991), Peters et al. (2002), Shuping et al. (2000), Sjögren et al. (1991), Waltimo et al. (2005) and Yared & Bou Dagher (1994). Eight studies that compared microbiological status of pre- and post-medicated human root canals were excluded for various reasons (Table 3).

Only one study included a small control group (12 teeth), in which canals were left empty (no intracanal medicament) between appointments (Waltimo et al. 2005). The remaining seven studies simply compared the frequency of positive cultures before and after calcium hydroxide medication.

Data summary of included studies

Sample size ranged from 18 to 60 teeth. None of the papers reported the rationale for selecting the sample size. Endodontic treatment procedures varied among studies in type of instrumentation technique, concentration of sodium hypochlorite used as irrigant, removal of smear layer, and the method and duration of placement of calcium hydroxide. Overall, the clinical procedures followed accepted standards, with the following exceptions: (i) Ørstavik et al. (1991) used saline rather than sodium hypochlorite as irrigant, thus omitting a major component of antibacterial action during canal debridement; (ii) Yared & Bou Dagher (1994) undertook minimal canal enlargement, such that all canals still had positive culture at S2; (iii) Only one study (McGurkin-Smith et al. 2005) reported removing the smear layer using EDTA; (iv) Peters et al. (2002) did not use a lentulo spiral to place calcium hydroxide in the canals. Sodium hypochlorite was used as an irrigant with concentration ranging between 0.5% and 5.25%. However, effects of different NaOCl concentrations on microbiological status have not been demonstrated clinically. Calcium hydroxide was used over different durations (1–4 weeks). Again, the duration of calcium hydroxide dressing seems to be inconsequential once a 1-week duration is reached (Sjögren et al. 1991).

Microbiological technique

Most of the studies followed strict protocols for pre-sample sterilization of the tooth surface (Möller 1966), although some of the studies deviated slightly from this. This deviation seemed, however, unimportant because all studies reported 0% or close to 0% positive cultures of pre-sample sterilization (control sample), i.e. sterilization protocols of the tooth surface before canal access were adequately effective. Exact procedures of
the initial bacterial sample prior to canal preparation (S1) varied from study to study, but S1 results showed 100% or close to 100% bacterial positive cultures in all studies. Canal preparation protocols were widely diverse, ranging from small master apical sizes (which in practical terms equates to minimal canal debridement) to unusually large master apical size. Premedication sample (S2) protocols also varied from simple (charcoal paper points used to absorb canal contents) to complex. Reflecting this variability, S2 results ranged from 14.3% to 100% positive bacterial cultures. Post-medication sample (S3) protocols were similar in all studies. Citric acid was generally used to neutralize calcium hydroxide and sampling techniques were repeated as in S2. S3 results ranged from 0% to 71.4% positive to bacterial culture.

**Meta-analysis**

Outcomes of individual studies and a summary of meta-analysis results are shown in Tables 4–5 and Fig. 1. Six studies demonstrated a statistically significant reduction in the number of bacteria-positive canals after medication, whilst two did not. Meta-analysis was performed on the combined data. The outcome measure was based on binary data, i.e. positive/negative bacterial cultures. A comparison was made between pre- and post-medication of the same root canals (matched samples or matched pair designs). Thus, McNemar’s test, which could take culture reversal into consideration by separately tracking canals with positive and negative cultures at S2, was performed on outcome measures (Moore & McCabe 2003). This test provided a significance level ($P$-value) for individual studies as shown in Table 6. Between-study heterogeneity was assessed using the standard chi-squared test or Q-statistic. The eight studies were heterogeneous ($\text{Test of Homogeneity Cochran } Q (\chi^2) = 111.93, \text{ df } = 7, \ P < 0.001$). Thus, random effect methods for combining study estimates were used and an overall estimate was produced. Risk differences of included studies were combined as generic inverse variance data type ($RD_{\text{Pooled}} = -21\%; 95\% \text{ CI: } -47\% \text{ to } 6\%$). The difference between pre- and post-medication was not statistically significant ($P = 0.12$). Thus, based on the current best available evidence, calcium hydroxide

<table>
<thead>
<tr>
<th>Citation</th>
<th>Sample size</th>
<th>S1 (%)</th>
<th>S2 (%)</th>
<th>S3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ørstavik et al. (1991)</td>
<td>23</td>
<td>22 (95.7)</td>
<td>13 (56.5)</td>
<td>8 (34.8)</td>
</tr>
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<td>18</td>
<td>18 (100)</td>
<td>9 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Yared &amp; Bou Dagher (1994)</td>
<td>60</td>
<td>60 (100)</td>
<td>60 (100)</td>
<td>19 (31.7)</td>
</tr>
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<td>Shuping et al. (2000)</td>
<td>40</td>
<td>39–40 (97.5–100)*</td>
<td>14–16 (35–40)*</td>
<td>3 (7.5)</td>
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<tr>
<td>Peters et al. (2002)</td>
<td>21</td>
<td>21 (100)</td>
<td>3 (14.3)</td>
<td>15 (71.4)</td>
</tr>
<tr>
<td>Kvist et al. (2004)</td>
<td>44</td>
<td>43 (95.6)</td>
<td>28 (63.6)</td>
<td>16 (36.4)</td>
</tr>
<tr>
<td>McGurkin-Smith et al. (2005)</td>
<td>27</td>
<td>25 (92.6)</td>
<td>14 (51.9)</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>Waltimo et al. (2005)</td>
<td>18</td>
<td>18 (100)</td>
<td>4 (22.2)</td>
<td>6 (33.3)</td>
</tr>
</tbody>
</table>

S1, bacterial sampling after initial access; S2, bacterial sampling after the cleaning and shaping procedure is complete (immediately before canal medication); S3, bacterial sampling when the canal is re-accessed 1–4 weeks later, after the medication has been removed.

*Exact data were not available after repeated attempts to contact authors.

<table>
<thead>
<tr>
<th>Citation</th>
<th>Sample size</th>
<th>S2+ → S3+</th>
<th>S2− → S3+</th>
<th>S2+ → S3−</th>
<th>S2− → S3−</th>
</tr>
</thead>
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<td>0</td>
<td>9</td>
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<tr>
<td>Yared &amp; Bou Dagher (1994)</td>
<td>60</td>
<td>19</td>
<td>0</td>
<td>41</td>
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<td>Shuping et al. (2000)</td>
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<td>0</td>
<td>11–13*</td>
<td>24–26*</td>
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<td>2</td>
<td>11</td>
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<td>Waltimo et al. (2005)</td>
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<td>0</td>
<td>6</td>
<td>4</td>
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</table>

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has limited efficacy in eliminating bacteria from human root canal when assessed by culture techniques.

Discussion

The level of evidence

Randomized-controlled clinical trials are high in the hierarchy of quality of evidence for determining therapeutic efficacy. These trials can establish the most convincing causal relationship (Greenhalgh 2006), because they minimize confounders and maximize control over the trial environment (Elwood 1998).

Even though seven of the eight included studies in this review were not randomized-controlled trials, the fact that the same roots were sampled before and after medication made them self-controlled (identical subjects for pre- and post-medicated samples) and the results provided were more efficient statistically because of the increased inference power. The eighth study (Waltimo et al. 2005) was a randomized prospective controlled trial that included a group of nonmedicated canals, but the numbers in each group were small and the data were part of a larger study (Trope et al. 1999), in which microbiological data were not described. Waltimo et al. (2005) did not report a significant benefit for calcium hydroxide medication compared with empty canals (chi-squared test with Yates correction; $P = 0.31$).

Publication bias

Publication bias is a tendency that some studies are less likely to be published if studies show nonstatistically significant results or if the results go against the prevailing theory. Publication bias could falsely skew the conclusion of meta-analyses in either direction. Funnel plots and rank correlation tests can determine if

<table>
<thead>
<tr>
<th>Citation</th>
<th>Sample size</th>
<th>Rate difference (%)</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>$P$-value</th>
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<td>-42</td>
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<td>-22</td>
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<td>0.752</td>
</tr>
<tr>
<td>Combined eight studies</td>
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<td>-21</td>
<td>-47</td>
<td>6</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Exact data were not available after repeated attempts to contact authors.
publication bias exists (Begg & Mazumdar 1994, Sutton 2000), however, at least 25 studies are required for these tests to be informative (Glasziou 2001). Therefore, the existence of publication bias can neither be confirmed nor denied in this review.

**Heterogeneity**

**Subject differences**
Patients from different geographical and ethnic backgrounds could have different composition of their oral microbial flora. This could be the result of either ethnic difference per se or related environment or even diet. Whatever the true cause of these differences, it has been shown that such differences in microbial flora do exist (Baumgartner et al. 2004, Umeda et al. 1998). Patients of included studies were drawn from the United States, Sweden, the Netherlands and Lebanon. This geographical difference could potentially result in differences of endodontic microbial milieu and hence susceptibility to calcium hydroxide.

**Canal preparation protocol differences**
Canal preparation protocols were diverse among the eight studies though generally within accepted clinical procedures. In one study, only maxillary central incisors were utilized (Yared & Bou Dagher 1994). They were prepared to only size 30 or 40; theoretically, many canals were likely to be inadequately enlarged. This may be the reason why post-canal preparation sample (pre-medicated sample or S2) was 100% positive to bacterial culture, which could spuriously inflate antibacterial efficacy of calcium hydroxide in comparison with other studies, where S2 was much lower than 100%.

**Method of calcium hydroxide placement**
A lentulo spiral was used to place calcium hydroxide in all but one study, in which calcium hydroxide was carried and plugged with the blunt end of a paper point (Peters et al. 2002). This method of placement has been shown in extracted teeth to be inferior to the lentulo spiral method, in terms of increasing the pH in dentine (Teixeira et al. 2005). The latter authors conjectured that the paper point placement method was unable to fill an entire canal, resulting in a lower pH value and reduced antibacterial efficacy. The study of Peters et al. (2002) was mainly responsible for the heterogeneity among the eight studies, and had it been excluded, meta-analysis would have shown a significant effect of calcium hydroxide. Exclusion was, however, not justified because the paper clearly stated that the completeness of placement of calcium hydroxide was confirmed radiographically.

**Outliers**
Outliers are observations that for some reason do not fit within the typical range of others. They can cause potential computational and inference problems, which could lead to distortion of estimates and P-value resulting in faulty conclusions. However, ignoring outliers or simply discarding them at will is not a good scientific approach (Barnett & Lewis 1994). Only as a last resort should outliers be deleted, if legitimate errors can be identified. In a general sense, Peters et al. (2002) and Waltimo et al. (2005) were outliers (Fig. 1), however, no methodological errors or biological reasons could be identified; as a result exclusion of these was not justifiable. In addition, these two articles were not statistically considered outliers as such. To determine whether any particular data set is an outlier, the inter-quartile-range (IQR) needs to be calculated. IQR is a range between first and third quartile. Any data set that lies in between third quartile +1.5 IQR and first quartile −1.5 IQR is not considered an outlier (Barnett & Lewis 1994). The data from Peters et al. (2002) and Waltimo et al. (2005) were within this range.

**Diagnostic accuracy of the microbiologic sampling technique**
Microbiological sampling of root canals is complex and its accuracy has been questioned (Molander et al. 1990). It has been hypothesized that this inaccuracy could stem from the anatomical complexity of the root canal system. Bacteria reside within dentinal tubules and in accessory canals, fins and other irregularities preventing bacteria from being easily retrieved by microbiologic sampling. Additionally, remnants of antibacterial medication may enter the sample, suppress growth at the laboratory and bring about a false-negative result (Reit et al. 1999). Calcium hydroxide by itself has been shown to compromise the sensitivity of microbiological sampling (Molander et al. 1990). Sensitivity was only 33% when canals had been dressed with calcium hydroxide. The present review has substantiated this view by clearly documenting culture reversals in four of the eight studies (Table 5). Thus, negative culture clearly does not equate to elimination of bacteria from the entire root canal system, which raises questions of the value of current microbiological sampling techniques (Wu et al. 2006).
Analysis of colony forming units (CFUs) would be useful additional information rather than positive or negative cultures as reported here; however, only four papers reported CFU, and then only either mean or median CFU. Unless the individual patient data are available, no detailed analysis is possible.

**Conclusion**

Based on the current best available evidence, calcium hydroxide has limited effectiveness in eliminating bacteria from human root canal when assessed by culture techniques. The quest for better antibacterial protocols and sampling techniques must continue to ensure that bacteria have been reliably eradicated prior to obturation.

**References**


Antibacterial efficacy of calcium hydroxide  

Sathorn et al.


