FT-Raman spectroscopy of calcium hydroxide medicament in root canals

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Abstract

Kwon TY, Fujishima T, Imai Y. FT-Raman spectroscopy of calcium hydroxide medicament in root canals. *International Endodontic Journal*, **37**, 489–493, 2004.

Aim To investigate chemical changes in calcium hydroxide introduced into human root canals as a medicament using Fourier transform-(FT) Raman spectroscopy.

Methodology Ten necrotic maxillary anterior teeth were selected in 10 patients. The teeth were divided into five treatment groups, according to the survey time. Root canal instrumentation was performed with hand instruments until the master apical file was size 40. Calcium hydroxide paste, in a 1 : 1.25 mixture by weight of powder and distilled water, was introduced directly into the root canal with a lentulo-spiral filler and then condensed with a finger plugger. The access cavity was sealed with a temporary dressing. After 2 and 4 days, then 2, 4 and 6 weeks, the calcium hydroxide paste was sampled with a K-file and then analysed using FT-Raman spectroscopy. The excitation source was an Nd : YAG laser with an excitation wavelength of 1064 nm. All spectra were taken with a laser power of 200 mW, 275–1185 scans, and 4 cm^{-1} resolution. The conversion of calcium hydroxide to calcium carbonate was calculated on the basis of the spectral data obtained from the mixtures of both compounds.

Results The calcium hydroxide paste in the apical region showed weak bands at 1088 and 284 cm^{-1}, in addition to bands associated with calcium hydroxide. The weak bands, assigned to calcium carbonate, became stronger with time. Calcium carbonate content increased rapidly in the first 2 days and then tended to increase slowly. Approximately 11% of the calcium hydroxide at the apical portion of the canal was converted to calcium carbonate after 6 weeks. However, little alteration of the paste was noticed in the samples from the middle portion of the canal.

Conclusions Calcium hydroxide medicament in root canals became transformed into calcium carbonate in the apical region within 2 days. Although the transformation continued with time, approximately 90% of the calcium hydroxide remained unchanged after 6 weeks.

Keywords: calcium carbonate, calcium hydroxide, FT-Raman spectroscopy, intracanal medication.

Received 9 December 2003; accepted 16 March 2004

Introduction

Calcium hydroxide is not categorized as a conventional antiseptic, but is clinically effective in eliminating micro-organisms from the root canal space (Byström *et al.* 1985, Safavi *et al.* 1985). The antimicrobial properties of calcium hydroxide are directly related to pH (Byström *et al.* 1985, Evans *et al.* 2002). Calcium hydroxide paste for intracanal use is normally a thick suspension of calcium hydroxide powder in water or saline. In a water suspension, less than 0.2% of the powder is dissolved into calcium and hydroxyl ions. Because it is a powerful alkaline, this results in a paste with a pH of approximately 12.5 (Ardeshna *et al.* 2002). This pH is sufficient to kill on contact most bacterial root canal species (Byström *et al.* 1985). The antimicrobial activity of calcium hydroxide is related to the release of hydroxyl ions in an aqueous environment

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(Safavi & Nakayama 2000). Therefore, maintaining a high pH over time is a prerequisite for effective use of calcium hydroxide paste as an intracanal medicament.

Calcium hydroxide is sometimes used as a long-term dressing or a short-term root filling if treatment cannot be completed for logistical reasons or if the tooth needs to be reviewed to assess the outcome of treatment. It is generally believed that the bacteria remaining after initial treatment can be controlled by an intracanal medicament such as calcium hydroxide (Byström *et al.* 1985). However, there is controversy as to whether the effect of calcium hydroxide in the root canal is long lasting. When calcium hydroxide paste comes into contact with carbon dioxide or carbonate ions, calcium carbonate is formed chemically (Holland *et al.* 1979), which may cause a drop in pH in the root canal, and, therefore, may reduce the antibacterial effectiveness. However, few studies have repeated the chemical changes of calcium hydroxide paste in the root canal, especially *in vivo*.

The purpose of this study was to investigate chemical changes of calcium hydroxide within medicated root canals, over time using Fourier transform-(FT) Raman spectroscopy. FT-Raman spectroscopy is a useful tool for the analysis of bone tissue and calcium phosphate compounds including hydroxyapatite (Sauer *et al.* 1994, Rehman *et al.* 1995, Wentrup-Byrne *et al.* 1997).

Materials and methods

Materials

The paste formulation used in this study was a 1 : 1.25 mixture by weight of pure-grade calcium hydroxide powder (Duksan Pure Chemical Co. Ltd, Ansan, Korea) and distilled water. Calcium carbonate was used as a reference material in the chemical analysis.

Samples *in vivo*

Ten maxillary anterior teeth (three central incisors, two lateral incisors and five canines) were selected in 10 systemically healthy patients. All selected teeth were necrotic, had not received endodontic treatment previously and showed radiographic evidence of periapical lesions. The teeth were divided into five treatment groups, according to the survey time (2 and 4 days; 2, 4 and 6 weeks). All subjects were informed of the possible complications of the procedure. Ethics committee approval was sought and granted, and informed written consent was obtained from each patient before treatment.

Access cavities were prepared with high-speed diamond burs under irrigation with sterile water. The working length was set at 0.5 mm short of the foramen with an electronic apex locator (Root-ZX, J. Morita, Tokyo, Japan). A radiograph was taken with the file in place at the length indicated by the locator. Root canals were instrumented with conventional K-type files, H-type files and reamers, until the master apical file was size 40. The canals were irrigated alternately with 3% hydrogen peroxide and 5.2% sodium hypochlorite. Irrigation was carried out with a disposable plastic syringe of 5 mL capacity with 25-gauge blunt needle to ensure the irrigant approached the apex.

Calcium hydroxide paste was made by mixing calcium hydroxide powder with distilled water to a creamy consistency at a powder : liquid ratio of 1 : 1.25. Each canal was dried with paper points, and then the calcium hydroxide paste was introduced directly into the root canal with a lentulo-spiral filler and then condensed with a finger plugger. A radiograph was taken to ensure proper placement of the calcium hydroxide paste in the root canal. The access cavity of the tooth was then sealed with a temporary dressing (Cavit, ESPE, Seefeld, Germany) with a minimum thickness of 3 mm.

After 2 and 4 days and 2, 4 and 6 weeks, the calcium hydroxide paste was sampled; after removing the temporary coronal restoration, the sample was carefully taken from the middle third of the root canal with a K-file, and the region was then cleaned with paper points. Following this, an apical sample was obtained from the apical region without contacting directly the root canal wall to avoid contamination throughout the canal. All endodontic procedures were performed by one clinician. The file was placed in sterile bag and then transferred to the laboratory within 30 min for spectroscopic procedures.

Spectroscopic analysis

The samples were analysed by FT-Raman spectroscopy. Raman spectra were recorded using a FT-Raman spectrometer (RFT-800, JASCO, Tokyo, Japan). The excitation source was an Nd : YAG laser with an excitation wavelength of 1064 nm. All spectra were taken with a laser power of 200 mW, 275–1185 scans (depending on the samples) and 4 cm^{-1} resolution. The samples were placed on a stainless steel plate and packed with a metal spoon prior to analysis. The laser

beam was focused on the sample surface and the acquired spectra were analysed using Jasco FT software for Windows (Version 1.20.00, JASCO). Raman spectra of calcium hydroxide, calcium carbonate, their mixtures and hydroxyapatite were also analysed as references.

Determination of the calcium carbonate content in calcium hydroxide samples

The Raman spectra of the samples and four kinds of standard mixture of calcium hydroxide and calcium carbonate were recorded. The intensity ratio for calcium carbonate was calculated from $I_{1088}/(I_{1088} + I_{796})$, where I_{1088} and I_{796} are the intensity at 1088 cm^{-1} for calcium carbonate and 796 cm^{-1} for calcium hydroxide. The plots of the intensity ratio against calcium carbonate content in the standard mixtures gave the calibration curve.

Results

Figure 1 shows the spectra of the samples in the apical region at 2 days and 6 weeks. The samples showed bands at 1088 and 284 cm^{-1}, in addition to bands associated with calcium hydroxide. The weak bands became stronger over the experimental period. The bands were attributed to calcium carbonate on the basis of reference spectra for calcium hydroxide and calcium carbonate (Fig. 2). Raman bands appeared at 930, 796, 721 and 363 cm^{-1} for calcium hydroxide and at 1088, 714 and 284 cm^{-1} for calcium carbonate. The Raman spectra of the samples obtained from the middle part of the root canal were nearly the same as calcium hydroxide, even after 6 weeks, suggesting that little calcium hydroxide converted to calcium carbonate.

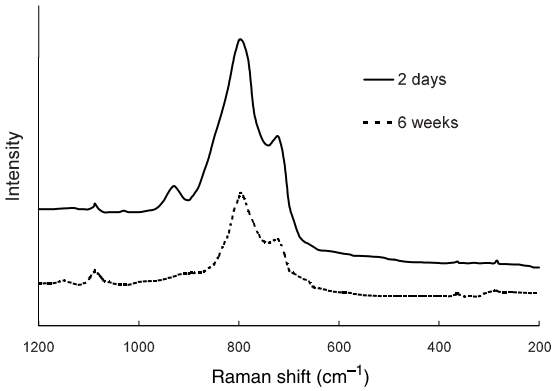

Figure 1 Spectra of the samples near the apical end at 2 days and 6 weeks.

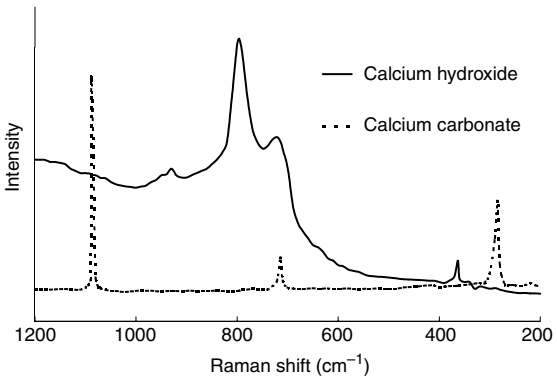

Figure 2 Reference spectra for calcium hydroxide and calcium carbonate.

Figure 3 shows plots of the intensity ratio for calcium carbonate against calcium carbonate content in the standard mixture of calcium hydroxide and calcium carbonate. The content of calcium carbonate converted from calcium hydroxide in the root canal was calculated using the calibration curve.

Figure 4 shows temporal changes in the content of calcium carbonate and calcium hydroxide in the calcium hydroxide samples retrieved from the apical region and the middle region of the root canal. Calcium carbonate content in the apical region increased rapidly within 2 days and then tended to increase slowly. Approximately 11% of the calcium hydroxide in the apical portion of the canal was converted to calcium carbonate after 6 weeks. However, little

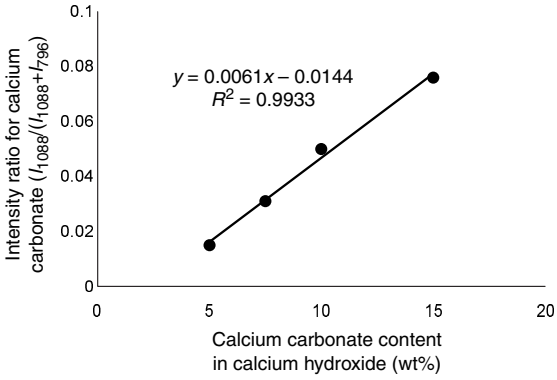

Figure 3 Plots of intensity ratio for calcium carbonate against calcium carbonate content in the standard mixture of calcium hydroxide and calcium carbonate.

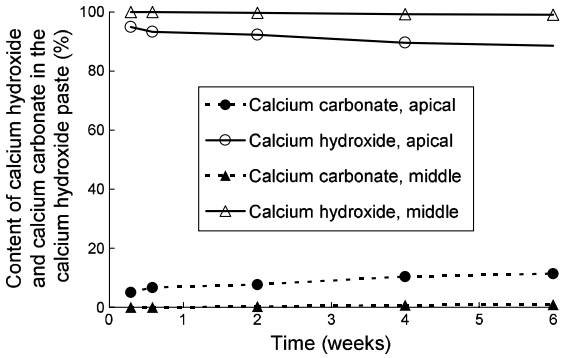

Figure 4 Temporal change of the content of calcium hydroxide and calcium carbonate in the calcium hydroxide samples retrieved from the apical end and the middle region of the root canal.

calcium carbonate was detected in the samples from the middle portion of the canal.

Discussion

Most endodontic pathogens are unable to survive in the highly alkaline environment provided by calcium hydroxide (Heithersay 1975). Several bacterial species commonly found in infected root canals are eliminated after a short period in direct contact with this substance (Byström *et al.* 1985). Therefore, maintaining a high pH over time is of importance for effective use of calcium hydroxide as an intracanal medicament. However, when calcium ions come into contact with carbon dioxide or carbonate ions, calcium carbonate is formed (Holland *et al.* 1979). This material has a very low solubility and has a pH of 8, and unlike calcium hydroxide, has neither biological nor antibacterial properties (Estrela 1994).

In this study, calcium carbonate formed from calcium hydroxide was verified by using FT-Raman spectroscopy *in vivo* (Fig. 1). Some of the calcium hydroxide paste in the apical region had been converted into calcium carbonate within 2 days. The bands attributed to calcium carbonate became stronger over the experimental period and thus the conversion of calcium hydroxide into calcium carbonate progressed with time. Solak & Öztan (2003) demonstrated the pH changes of different calcium hydroxide mixtures. In their study, the pH value of the calcium hydroxide mixed with distilled water rose to pH 12.2 and decreased to pH 11.5 after 48 h, then stabilized after 7 days. The pH change or decrease of calcium hydroxide paste medicament in the root canal system seems to occur in the short term as a result of the instant conversion of that material into calcium carbonate.

The hydroxyl concentration may be decreased and its antibacterial effectiveness may be reduced or impeded (Siqueira & Uzeda 1998, Siqueira *et al.* 1998) by the action of a buffering system (bicarbonate and phosphate), acids, proteins and carbon dioxide. The main natural agent capable of influencing the pH is carbon dioxide. Fuss *et al.* (1996) reported intracanal pH changes of calcium hydroxide pastes exposed to carbon dioxide *in vitro*. They concluded that the pH of the pastes in teeth exposed to carbon dioxide was significantly reduced after 30 days, but after 30 days of exposure to carbon dioxide, they still maintained a purportedly bactericidal pH within the root canal. Calcium carbonate may originate from bacterial metabolism in the canal or dentinal tubules, or from surrounding tissues. Therefore, seepage of interstitial fluid through the apical foramen or accessory canals may serve as a source of that gas in the root canal. Excluding the possibility of coronal leakage, loss of efficacy may be caused by dilution with periapical fluids, especially in canals with wide apical foramina. An additional cause of reduction in pH of the paste may be neutralization by carbon dioxide dissolved in body fluid that penetrates through the apical foramen.

In the present study, the conversion of calcium hydroxide into calcium carbonate progressed with time, but was slow and approximately 90% of the calcium hydroxide remained unconverted after 6 weeks (Figs 1 and 4). It seems conceivable that carbon dioxide reacted with calcium hydroxide at the apical foramina and at accessory canal orifices, producing a plug of calcium carbonate that prevented further penetration of carbon dioxide into the root canal space. Moreover, calcium hydroxide has low solubility in water. As a result, in the moist environment of the root canal system, undissolved calcium hydroxide in a paste-like suspension will steadily dissolve, resulting in a sustained pH effect.

In contrast to the apical region, calcium hydroxide medicament in the middle portion of the root canal remained unchanged during the whole experimental period up to 6 weeks (Fig. 4). Little calcium carbonate was detected in the samples from the middle portion of the canal. Cohen & Lasfargues (1988) demonstrated that the transformation of calcium hydroxide to calcium carbonate occurs extremely slowly in closed containers, and only 1–2% had converted after several months. In open containers, however, approximately

30% had converted. The hydroxyl ions cannot easily penetrate the dentine because of the buffering capacity of hydroxyapatite (Wang & Hume 1988). Loss of material by diffusion through the dentinal wall would be expected to be minimal because of the selective permeability of dentinal tubules to hydroxyl ions. As a result of these characteristics of the dentine, little conversion of calcium hydroxide in the middle portion of the root canal into calcium carbonate seems to occur with time.

From the clinical perspective, the results of the present study suggest that calcium hydroxide as an intracanal medicament does not lose activity within a short period. Calcium hydroxide paste may allow for longer inter-visit periods in endodontic therapy, although these conclusions are based on a limited number of teeth.

Conclusions

The present study revealed the chemical change of calcium hydroxide medicament in root canals into calcium carbonate using FT-Raman spectroscopy *in vivo*. Calcium hydroxide was converted partly into calcium carbonate in the apical region within 2 days. This conversion progressed with time, but was slow and approximately 90% of the calcium hydroxide remained unchanged after 6 weeks. Little calcium carbonate was detected in the samples from the middle portion of the canal even after 6 weeks.

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