In Vitro Study of Intradentinal Calcium Diffusion Induced by Two Endodontic Biomaterials

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The aim of this in vitro study was to assess intratubular calcium penetration induced by two root canal restoration materials, one calcium oxide based, and the other calcium hydroxide based. Pig teeth were restored with no preliminary root canal preparation. The filling materials were left in place for 8, 15, or 21 days. The samples were then examined using various microanalytical techniques and, in parallel, by backscattered electron image (BEI) scanning electron microscopy. The Ca/P ratios obtained by microanalysis were higher for samples restored with calcium oxide. In addition, the distances over which the ratios increased were also greater than those obtained using calcium hydroxide. BEI photographs confirm these results and show corresponding retrodiffusion fringes.

Calcium hydroxide based materials are frequently used as temporary endodontic medications to sanitize the endodontium during root canal treatment (1) and to induce apicogenesis and apexification (2). Several properties of calcium hydroxide have led to the use of such materials: high pH, diffusion of OH⁻ ions across the dentine leading to gradual pH changes (3), and the diffusion of Ca²⁺ ions from the material into the dentine (4).

During a previous study (5), we demonstrated the usefulness of calcium oxide based materials. They increase filling hermeticity by consistently penetrating tubules and reducing the dentine-material interface to minima, even with no root canal preparation. Calcium oxide-based materials change into calcium carbonate in the presence of carbon dioxide produced by protein degradation and into calcium hydroxide on contact with residual water (5), suggesting that they may have properties similar to calcium hydroxide.

The in vitro study reported here has allowed us to compare calcium penetration into the dentine induced by calcium hydroxide and calcium oxide.

MATERIALS AND METHODS

The study was carried out using single-rooted lower teeth from pigs. The animals were 6 months old, which corresponds to the prepuberty period for pigs. This allowed us to obtain homogeneous lots as the animals were of the same race, the same age, and were sacrificed on the same day. Furthermore, pig teeth are ultrastructurally identical to human teeth. After extraction and transversal sectioning of the crown, the teeth were depulped and placed at 4°C in distilled water containing antibiotics (100 IU/ml of penicillin and 100 μg/ml of streptomycin).

Two materials were tested: a calcium hydroxide preparation obtained by mixing pure calcium hydroxide powder (0.9%) with sterile distilled water and a preparation containing 88% heavy calcium oxide and 33.3% zinc oxide in 80% glycolated water.

The teeth were divided into three homogenous groups of nine teeth: a control group (A), a calcium oxide group (B), and a calcium hydroxide group (C). They received no root canal instrumentation apart from depulping. The materials were inserted using a root canal paste carrier under sterile conditions in a laminar flow cabinet. Both ends of the teeth were then temporarily sealed using a zinc oxide/calcium sulphate cement without eugenol. The samples were incubated separately in a humidified container in an anaerobic chamber (FORMA Scientific; 1028, 80% N₂, 10% H₂, 10% CO₂). Three teeth from each group were incubated for 8, 15, or 21 days. After the incubation period, the samples were embedded in epoxy resin that was then polished to expose the root canal containing the material. Sheets of increasingly fine silicon carbide paper (220, 330, 500, and 1200 grain) were used. Final polishing was carried out using 3 μm diamond cloths to obtain perfectly smooth surfaces. The samples were then cleaned in alcohol and dehydrated with a 20 μm layer of colloidal gold. A Jeol JSM 35F scanning electron microscope equipped with a Tracor Northern TN 2000 system was used to analyze the samples at 15 Kv. This apparatus allows the detection of all elements with an atomic number superior or equal to 4 with a relative accuracy of 1% in a 1 μm² volume. A semi-quantitative analytical computer program (Standards) was used to determine the concentrations of the various elements detected. The concentration values were refined by iterative calculation (ZAF program) taking into account absorption and fluorescent interactions between the various elements. The elements chosen for identification were calcium, phosphorus, magnesium, and zinc. Each sample was analyzed centripetally at 6 points on an "xy" axis spread out as follows:

- Point 1: 400 μm from the outside root surface
- Point 2: 600 μm from the outside root surface
- Point 3: 600 μm from the root canal wall
- Point 4: 400 μm from the root canal wall
RESULTS

The averages of the analyses made on the three sample groups were plotted on curves showing the variation in Ca/P ratio as a function of distance for each incubation period. Magnesium and zinc concentrations did not vary.

After 8 days of incubation, the Ca/P ratio increased for groups B and C compared to control group A (Fig. 2), which showed little variation (1.3 to 1.4). The ratio for group B samples increased progressively from 1.5 at 40 to 60 µm from the root canal wall to 2.85 in the parietal zone (Fig. 2). The increase in group C Ca/P ratios began at 30 µm from the root canal wall and reached a maximum of 2.19 in the parietal zone (Fig. 2). BEI photographs confirmed these results, showing 54 µm retrodiffusion fringes for group B samples (Fig. 3) and 30 µm fringes for group C samples (Fig. 4).

After 15 days of incubation, the Ca/P ratios had increased significantly in group B. The ratio was 1.64 at 110 µm from the root canal wall and 2.37 in the parietal zone (Fig. 5). On the other hand, the ratio for group C was 1.62 at 70 µm from the root canal wall and 2.30 in the parietal zone (Fig. 5). BEI observations confirmed these results, showing 120 µm retrodiffusion fringes for group B samples (Fig. 6). The photographs also show evidence of intratubular penetration of calcium oxide based material beyond the retrodiffusion zone (Fig. 6). The fringes were, however, only observed over a 50 µm range when the teeth were filled with the calcium hydroxide material (Fig. 7).

After 21 days, average group B Ca/P ratios were even higher over a greater distance, reaching 1.79 at 130 µm from the root canal wall and 2.72 in the parietal zone (Fig. 8). In group C, the increase in the Ca/P ratio was even more marked than after 15 days, without, however, increasing in distance. The ratio was 1.79 at 40 µm from the root canal wall and 2.56 in the parietal zone (Fig. 8). BEI examination of group C samples showed a 60 µm
retrodiffusion fringe near the root canal wall (Fig. 9) compared to a 160 µm fringe for group B samples (Fig. 10).

For all three groups and incubation periods, the Ca/P ratio remained constant in the proximal dentine at the outside edge of the root, which was a control zone. BEI observations of calcium oxide filled samples indicated a significant decrease in retrodiffusion over time by the filling material.

**DISCUSSION**

Ca$^{2+}$ and OH$^-\,$ ions are liberated from the calcium hydroxide based material in a controlled and progressive fashion providing a therapeutic effect (2). The study by Deardorf et al. (4) measuring extraradicular Ca$^{2+}$ ion concentrations showed that dentine became permeable to Ca$^{2+}$ ions after 7 days. Troststatt et al. (3) demonstrated the presence of intradental pit changes in monkeys after restoration with calcium hydroxide. The pH was strongly alkaline in parietal zones and neutral in the peripheral dentine. Troststatt’s conclusions were confirmed by Fass et al. (7) who observed an increase in extraradicular pH after restoration with calcium hydroxide. Stehle et al. (8) demonstrated the presence of variable zones of alkalinity in intraradicular dentine that depended on the filling material used.

Our study substantiates the above observations. Microanalyses of the samples restored with either calcium oxide or calcium hydroxide based materials indicated that they all experienced an increase in Ca/P ratios. BEI examinations of the same samples confirmed this and showed the presence of a parietal band where calcium had penetrated into the dentine. It should be noted that this penetration was a highly regular phenomenon around the circumference of the root canal and involved the entire intradental parietal space. The diffusion was much deeper and more intense (Ca/P ratios) when the samples were restored with calcium oxide based materials.

Calcium diffusion also occurred in the unmineralized extracellular matrix present on the root canal walls of samples that had received no parietal mechanical instrumentation or chemical treatment. Retrodiffusion images indicated the presence of calcium deposits in this organic material that reduced the tooth/material interface, especially when the calcium oxide based material was used. In this group, the decrease in retrodiffusion of the material in the root canal indicated a transfer of calcium into the tooth.
calcium phosphate is a precursor to hydroxyapatite formation. Octocalcium phosphate has a characteristic crystalline rosette formation visible by scanning electron microscopy. We frequently observed similar intratubular crystalline formations in our study when calcium oxide based materials were used but less so for calcium hydroxide based materials. This has led us to hypothesize that intratubular precipitation of octocalcium phosphate enhanced the positive retrodiffusion zones we observed.

In summary, our study confirms that Ca²⁺ ions diffuse out of calcium oxide and calcium hydroxide materials. However, calcium oxide preparations, which also appear to possess properties specific to calcium hydroxide, enhance intratubular penetration, decrease the interface and promote the transfer of calcium from the filling material to the radicular dentine. These results have clinical significance and may lead to new endodontic treatments.

We thank for technical assistance J.C. Jegadin, J. Latyntj, P. Pallier, and C. Vulcalin for translation. G. Bourguignon. This study is supported by “Fondation Langlois” and “Consell Régional de Brittany” (France).

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References