Calcium release and pH-characteristics of calcium hydroxide plus points

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Abstract

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Aim To evaluate calcium ion release and pH-characteristics of calcium hydroxide plus points (CHPP), conventional calcium hydroxide points (CHP, both Coltène/Whaledent, Langenau, Germany) and aqueous calcium hydroxide suspension (CHS) (Calxyl, OCO, Dirmstein, Germany).

Methodology Ten CHPP or CHP of size 50 were immersed into 5 mL isotonic sodium chloride solution. Conventional $Ca(OH)_2$-free gutta-percha points served as negative control. Calcium release was measured up to 44 days by means of complexometric titration. Time dependent pH behaviour of all points in comparison with CHS was determined immersing 30 points of size 50 into 2.3 mL 0.9% wt NaCl-solution at time intervals of 0.5–72 h by a microelectrode measuring chain and a pH-meter. The surface morphologies of new and used gutta-percha points were evaluated qualitatively under a scanning electron microscope. Statistical evaluation was carried out using Kolmogorov–Smirnov-tests, Mann–Whitney-tests and multifactorial ANOVA.

Results For CHPP, a threefold greater calcium release was measured compared with CHP. Both types of points as well as CHS showed a maximum pH of approximately 12. Differences between groups were statistically significant for calcium release and pH (multifactorial ANOVA; $P < 0.001$). Both types of points showed porous surfaces after usage, with a rougher surface for CHPP.

Conclusions CHPP and CHP increased the pH of isotonic sodium chloride >11 within 3 min. CHPP had a greater release of Ca^{2+} compared with CHP.

Keywords: calcium hydroxide plus points, calcium hydroxide points, calcium release, gutta-percha, intracanal medication.

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Introduction

Calcium hydroxide was introduced by Hermann (1920) for treatment and filling of root canals. It was commonly used in Scandinavian dentistry in the 1950s as a pulp capping medium, based upon extensive research (Nyborg 1955). Later, it was empirically used in root canal treatment. In comparative clinical investigations, $Ca(OH)_2$ has been shown to behave in a superior manner compared with other substances (Byström *et al.* 1985, Safavi *et al.* 1985) and to be well-tolerated by patients (Gambarini 1991). The beneficial effect of $Ca(OH)_2$ has been related to the break up of the fatty acids of the gram-negative bacterial cell membrane by Ca^{2+} together with the hydroxyl ions (OH^-) (Safavi & Nichols 1993, 1994), the inactivation of bacterial endotoxin (Tanomaru *et al.* 2003) and by damage to bacterial DNA (Siqueira & Lopes 1999).

The most common and effective type of calcium hydroxide is an aqueous suspension (CHS) (Staehle *et al.* 1989, 1995). Despite its good antimicrobial effect (Byström *et al.* 1985, Sjögren *et al.* 1991, Siqueira & Lopes 1999, Hauman & Love 2003) it exhibits poor handling properties (Ricucci & Langeland 1997, Lambrianidis *et al.* 1999) and its distribution throughout the entire canal is problematic. Inserting an additional gutta-percha point into the root canal or delivering $Ca(OH)_2$ using a syringe system has potential

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advantages (Staehle *et al.* 1997). Another challenge is the complete removal of the suspension as demanded by the ESE-guidelines (European Society of Endodontology 1992). Lambrianidis *et al.* (1999) reported that 25–40% of the root canal wall was contaminated with CHS depending on the method of removal. Presence of residual CHS can impede the properties of endodontic sealers (Kim & Kim 2002) or its distribution into lateral canals (Goldberg *et al.* 2002). Recently developed endodontic points containing $Ca(OH)_2$ have been introduced to address the handling and application of conventional CHS.

In the course of a prospective clinical study, the calcium containing gutta-percha points (CHP) (Coltène/Whaledent, Langenau, Germany) were found to be as clinically successful as CHS (Ebert *et al.* 1998) when used as short-term medication. However, based on findings of other studies it was shown that the capacity of CHP to provide active ions is limited and that CHP can alkalize dentine or maintain alkaline pH within the root canal for approximately 1 week (Ardeshna *et al.* 2002, Azabal-Arroyo *et al.* 2002, Barthel *et al.* 2002).

Recently, new $Ca(OH)_2$ gutta percha points have been introduced additionally containing sodium chloride and tensides [Calcium Hydroxide Plus points (CHPP), Coltène/Whaledent]. In contact with aqueous solutions these additional components are dissolved, preparing the way for water to penetrate into deeper layers of the gutta percha point. This is meant to maintain the pH high over a longer period of time and also to enhance the wettability of the adjacent canal surfaces.

When using calcium hydroxide as an intracanal medication the vehicle plays an important role. It determines the ionic dissociation kinetics by causing $Ca(OH)_2$ to be solubilized and resorbed or adsorbed at various rates in the periapical tissues and within the root canal. It is necessary to investigate whether any added component may change the properties of the calcium hydroxide formulation (Gambarini 1993, Pacios *et al.* 2003).

The aim of this study was to investigate time-dependent calcium ion release and pH-characteristics of different gutta-percha points containing calcium hydroxide compared with calcium hydroxide suspension (CHS).

Materials and methods

Materials

Three different types of size 50 gutta-percha points were selected for the laboratory tests: gutta-percha points (GP), calcium hydroxide points (CHP) and CHPP (all Coltène/Whaledent). The detailed chemical compositions of the points are shown in Table 1.

The zinc oxide content of approximately 68% wt in conventional GP (Friedmann *et al.* 1975, Marciano & Michailesco 1989) is replaced by approximately 50% wt $Ca(OH)_2$ in CHP points (Table 1). CHPP points additionally contain tensides and sodium chloride. Calxyl (OCO-Präparate, Dirmstein, Germany), an aqueous CHS was selected as a control.

Calcium release

Standard solutions of 0.9% wt NaCl (Pharmacia & Upjohn GmbH, Erlangen, Germany), 25% wt ammonia, ethylenediamine-tetraacetate (EDTA) disodium salt (Titriplex® III) and Eriochrome® Black T (Merck KGaA, Darmstadt, Germany) were used for analysis. Titration solution consisted of 100 mL aqua bidest, 1 mL ammonia solution (25%) and 50 μL colour indicator (Eriochrome® Black T). Solutions with released $Ca(OH)_2$ were added and titrated with 0.001 mol L^{-1} Titriplex III (The Merck Index 1984).

Ten gutta-percha points in each group were fully immersed into 5 mL 0.9% wt NaCl solution to protect them from the acidic influence of atmospheric carbon dioxide and stored at 37 °C. Calcium release was measured after 0.5, 1, 2, 6, 12, 24 and 48 h in the same solution. Experiments were repeated three times for each group.

To determine the amount of released calcium, the specimens were subjected to complexometric titration using 0.001 mol L^{-1} aqueous EDTA solution and Eriochrome® as colour indicator. Based on the colour change from red into green the amount of released

Table 1 Ingredients of different gutta percha points and Calxyl (wt.%) as stated by the manufacturers

Ingredients (%w/w)	Gutta percha points	Calcium hydroxide points	Calcium hydroxide plus points	Calxyl (blue)
Gutta-percha	27–30%	40–42%	35–37%	–
$Ca(OH)_2$	–	54–56%	52–54%	23%
ZnO	68%	–	–	–
NaCl	–	–	+	–
Surfactant	–	–	5–10%	–
Pigments	+	+	+	–
$BaSO_4$	+	–	–	27%
Wax and oil	+	+	+	–
Water	–	–	–	50%

calcium was calculated. 1 mL 0.001 mol L^{-1} Titriplex III solution equals 40.08 μg calcium.

The accumulated calcium release was determined by immersing 10 points of each group into 5 mL 0.9% wt NaCl solution of 37 °C. Calcium release (three experiments per group) was measured after 1, 7, 15, 30 and 44 days. The solution was replaced after each measurement. After 44 days the surfaces of the points in each of the three groups were compared with those of untreated points: representative samples were evaluated in terms of surface morphology. The points were dried, mounted on SEM-stubs, gold-sputtered (Balzers SCD 040, Balzers, Liechtenstein), and analysed in a SEM (Leitz ISI SR 50, Akashi, Japan) at 2000-fold magnification.

pH-measurement

A measuring chain consisting of a microelectrode (Inlab 423; Mettler-Toledo GmbH, Giessen, Germany) and pH-meter (WTW Corp., Weilheim, Germany) was used. The tests were carried out in 2.3 mL 0.9% wt NaCl-solution at 37 °C whilst stirring with a magnet-rod. After adjusting the test conditions 30 points of size 50 were placed into the solution. The pH was determined after 3 min and 1, 6, 12, 24, 48 and 72 h, respectively. Five experiments were conducted for the two $Ca(OH)_2$-containing gutta-percha groups, two experiments for the conventional GP. In addition, the pH-value for the CHS was determined.

Statistical analysis

Statistical analysis was carried out using SPSSWin® 11.0 (SPSS Co., Chicago, IL, USA) using the Kolmogorov–Smirnov-test for one sample, Mann–Whitney-test and multifactorial ANOVA.

Results

Calcium release

The measurement of calcium release over 48 h (Fig. 1) showed similar behaviour for CHP and CHPP: a logarithmic release during the first 6 h was followed by a constant release rate up to 48 h. Calcium accumulation measurements indicated a further release up to 44 days. The delivery of CHP was constant for each interval (4–5 μg mg^{-1} points), whereas for CHPP greater amounts and a time-dependent increase of delivery from 10 to 23 μg mg^{-1}

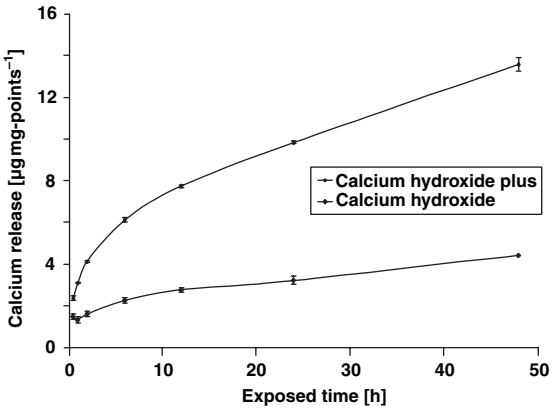

Figure 1 Calcium release of $Ca(OH)_2$-containing gutta percha points (48 h).

points were observed (Fig. 2). The measurements of GP (negative control) were slightly above zero due to the fact that the measurement method was not able to distinguish between Ca^{2+} and Zn^{2+}.

pH-measurement

The pH-curves for the three different points and for CHS are shown in Fig. 3. A rapid increase near to maximum saturation within the first 3 min was noted for both $Ca(OH)_2$-containing points [pH 11.8 (CHP); pH 12.0 (CHPP)]. These values were close to the pH-value for a saturated aqueous $Ca(OH)_2$-solution of 12.4 at 25 °C (Merck & Company, Inc. 1984). Consequently, the pH-value for the CHS was determined to be 12.5. The

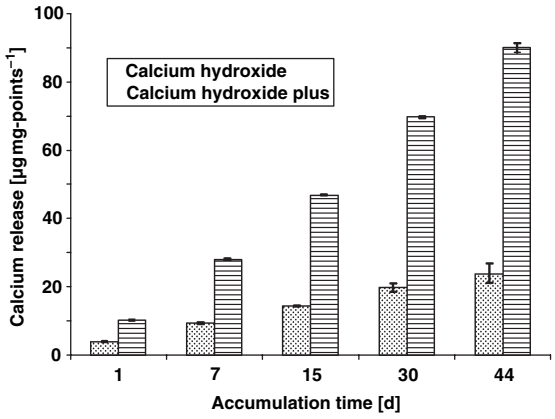

Figure 2 Accumulated calcium release of $Ca(OH)_2$-containing gutta percha points (44 days).

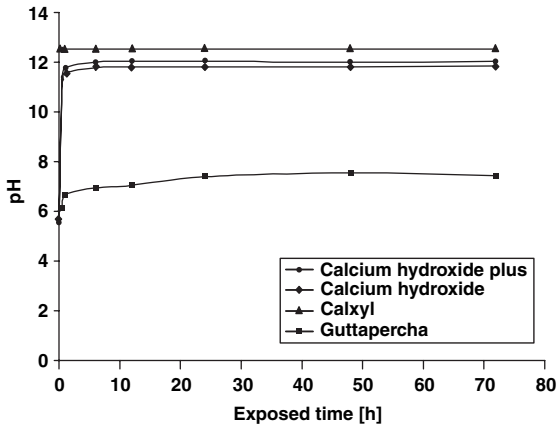

Figure 3 pH-development of different gutta percha points and Calxyl (72 h).

GP points serving as a negative control exhibited a neutral pH of 7–8.

Microscopic analysis

Microscopic evaluation of the untreated and leached surfaces of the points exhibited a comparable outcome: superficial particles in contact with the surrounding aqueous medium were dissolved over time, leaving voids within the surface of the gutta-percha points. Figures 4–7 clearly reveal a distinct pit corrosion-like mechanism on the surfaces, thus increasing the reactive surface area. CHPP exhibited a rougher and more porous surface structure in comparison with CHP.

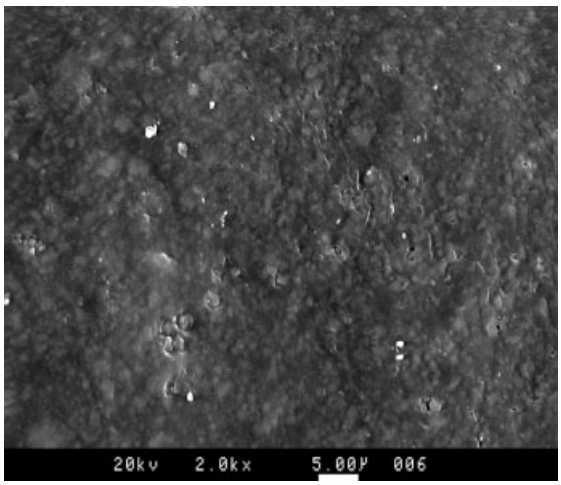

Figure 4 SEM-micrograph of a fresh calcium hydroxide point.

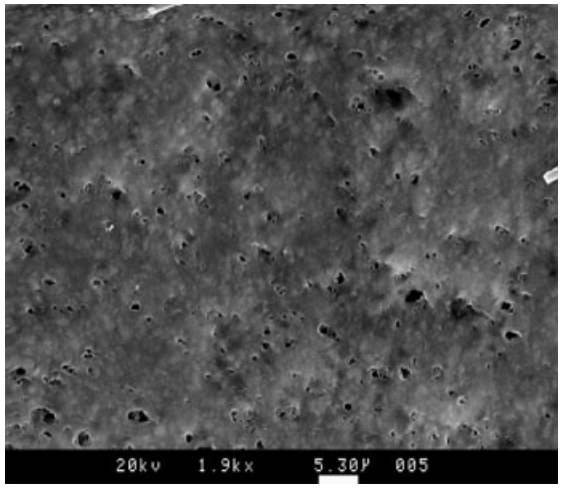

Figure 5 SEM-micrograph of a calcium hydroxide point after 44 days immersion in 0.9% NaCl solution.

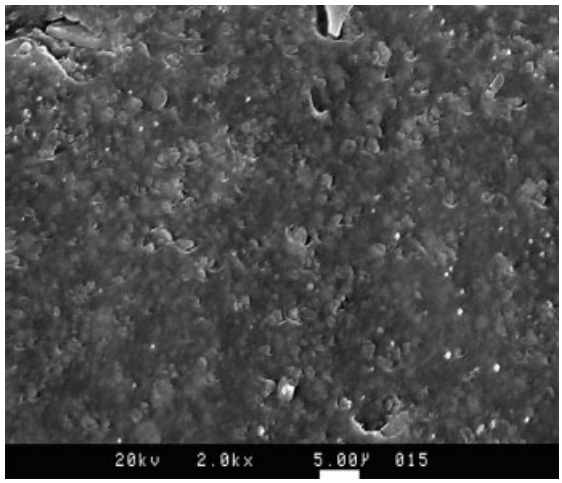

Figure 6 SEM-micrograph of a fresh calcium hydroxide plus point.

Statistical analysis

All groups were either normally distributed (Kolmogorov–Smirnov-test, $P > 0.05$) or exhibited identical values for the different measurements. Thus, multifactorial ANOVA could be performed. Calcium release, the accumulative calcium release and pH was time dependent and the groups were significantly different (multifactorial ANOVA, $\mathbf{P} < 0.001$). Due to relatively small standard deviations, even slight differences (e.g. pH 11.8 for CHP versus pH 12.0 for CHPP) could be

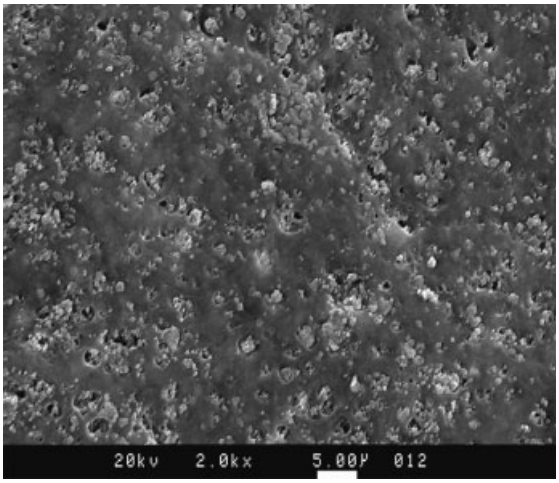

Figure 7 SEM-micrograph of a calcium hydroxide plus point after 44 days immersion in 0.9% NaCl solution.

identified as significantly different (Mann–Whitney-test, $P < 0.05$).

Discussion

The increased calcium release within the CHPP group (Figs 1 and 2) may be attributed to an enhanced dissolution from the surface of the point. The highly water-soluble components of the tenside and sodium chloride incorporated in CHPP are likely to be responsible for the surface corrosion and consequent improved calcium release. After superficial particle dissolution in CHPP, a larger surface accounts for further dissolution. This mechanism might be responsible for the increasing calcium release over time observed for CHPP. SEM analysis supported an increased release of elements from the CHPP since a greater number and size of residual void was found on the leached CHPP surface compared with CHP (Figs 4–7). The tenside reduces the surface stress of liquids followed by a more efficient calcium penetration into the dentine fluid. This effect might be the reason for an enhanced alcalization of outer dentine by CHPP compared with Calxyl up to 7 days (Ho *et al.* 2003).

The analytical titration method detected zinc ions (Zn^{2+}) released from ZnO. According to previous investigations (Bohnwagner 1997) the amount of released Zn^{2+} from gutta-percha was only in the range of 50–60 PPM (14 days) which is approximately 0.02 µg Zn mg^{-1} point. In agreement with this former study, the ion release of gutta-percha was rather low compared with the test groups. ZnO exhibits some antibacterial activity due to the cell toxicity of Zn^{2+}-ions (Moorer & Genet 1982, Silver 1996, Bohnwagner 1997, Podbielski *et al.* 2000).

The present study could elucidate a threefold higher Ca^{2+} ion release from CHPP compared with CHP. The new points also showed a slightly superior pH value. The pH of CHP, CHPP and CHS was found to be in the range of pH 12. In contrast Ferreira *et al.* (2004) found that CHP was not able to raise the pH of the surrounding medium above pH 7. They also reported a pH for a CHS of only pH 11.3. These differences might be because of different analytical methods. For calcium release measurements, values for CHPP were reported to be about one-third compared with CHS but above the values of an oily calcium hydroxide preparation (Ferreira *et al.* 2004).

In order to precisely assess the amount of released calcium, the accuracy of the applied technique is of great importance. Methods such as atomic absorption spectrometry, spectrophotometry, fluorometry, flame photometry or complexometric titration with EDTA are all suitable measurement techniques (Robertson & Marshall 1979, Economides *et al.* 1999). However, there is a lack of comparability between results from the different methods used because of the great variability of experimental conditions and materials tested (Economides *et al.* 1999).

The pH can either be determined by atomic absorption spectrophotometry or by microelectrode potentiometry (Tagger *et al.* 1988, Economides *et al.* 1999). Complexometric titration as well as the potentiometric pH detection was chosen because of the ease of experimental procedure, high accuracy and reproducibility of the methods (Cooper 1977, Robertson & Marshall 1979). The experiments were carried out in isotonic 0.9 wt% NaCl solution to ensure a minimum ionic conductivity of the reference solution. Buffered suspensions on the other hand might result in varying release kinetics from different materials (Tagger *et al.* 1988).

Azabal-Arroyo *et al.* (2002) reported that the alkalizing capacity of CHP within the root canal *in vivo* is complete within 7 days. Another *in vivo* study on CHS observed conversion of calcium hydroxide into calcium carbonate to be 11% of the original amount of calcium hydroxide at the apical part of the root canal after 6 weeks (Kwon *et al.* 2004). Further research has to be conducted to establish the amount of available calcium hydroxide necessary to act against the buffer capacity of dentine and tissue fluids in terms of intracanal

medication to maintain an effective antimicrobial action. However, acceptable clinical results have already been shown for CHP (Ebert *et al.* 1998), and thus might be employed for an improved ionic release profile.

Conclusions

Within the limits of this study it can be concluded that CHPP release a threefold amount of calcium ions compared with the customary CHP under laboratory conditions.

Acknowledgements

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