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An *in vitro* study of the pH of three calcium hydroxide dressing materials

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Abstract – Calcium hydroxide is widely used as a root canal dressing material because of its favorable alkalinizing effect. It has been suggested that the action comes from diffusion of hydroxyl ions through the apical foramen. The purpose of this *in vitro* study was to test the pH changes that occurred over a period of 30 days using a mixture of calcium hydroxide and distilled water and two commercial calcium hydroxide products in a simulated periapical environment. The materials were inserted in glass tubes closed at one end, which were placed in individual vials containing distilled water at a pH 7.4. Unfilled glass tubes were used as controls. Alkalinity changes of the medium were measured at 1 and 24 h and 15 and 30 days. The alkalinizing properties of all materials showed a rapid increase at 1 and 24 h followed by a continuous but more gradual increase from 15 to 30 days. The control tubes did not cause a change in pH of the medium, which remained at pH 7.4. At the end of the observation period, the alkalinizing properties of Calasept and Ultracal XS were significantly higher ($P < 0.05$) than the calcium hydroxide/distilled water paste.

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Calcium hydroxide is the preferred material for an intracanal dressing because of its favorable antimicrobial action (1, 2). It is also effective in arresting inflammatory root resorption (2–4) and promotes the reparative process of periapical tissues resulting in the formation of hard tissues (2, 5, 6). Furthermore, its use as an interim dressing has also been advocated (2, 7). One of the main benefits of calcium hydroxide-based materials is the high pH they impart on the surrounding environment; however, the exact mechanism of its action is not fully understood (2). When calcium hydroxide is used as a root canal dressing, the increase in pH in the surrounding tissues results mainly from the release of hydroxyl ions. The available hydroxyl ions at the site of application constitute one of the most important factors and accounts for its efficacy. Therefore, hydroxyl ions must be able to diffuse from the material through the dentinal tubules or the apical foramen to reach surrounding tissues. To be effective, calcium hydroxide has to be adequately placed and condensed into the root canal space, usually in combination with a carrying vehicle (8). Different techniques have been described (8–10); however, in most instances these are difficult to execute, especially in cases with narrow and curved root canals. To solve this problem, commercially available formulations of non-setting calcium hydroxide-containing pastes have been introduced as temporary intracanal dressings. Their use has an advantage over chair side prepared pastes in that they have a creamy consistency and can be easily placed with a 27- or 30-gauge needle. These non-setting preparations are generally composed of calcium hydroxide in a saturated aqueous solution to which additional components have been added that influence the viscosity and/or contrast. As these materials have proprietary chemical compositions, it is of interest to investigate the alkalinizing ability of each compound. The purpose of this preliminary report is, therefore, to assess *in vitro* the pH characteristics of three non-setting calcium hydroxide preparations in a simulated periapical environment.

Materials and methods

For this study, 17.0-mm-long glass tubes (Oscar de Lucca SA, Buenos Aires, Argentina) closed at one end and with an internal diameter of 1.0 mm were used. The tubes were cleaned with 1% hydrochloric acid followed by rinsing with distilled water (pH 7.4) to remove all contamination. After cleaning, they were autoclaved. For each experimental material 20 tubes were used ($n = 20$). In Group 1 the tubes were filled flush with a paste prepared by mixing calcium hydroxide powder (Farmadental, Buenos Aires, Argentina) and distilled water with a pH of 7.4, in a powder/water weight percent ratio of 50%. The paste was introduced into the tubes using a lentulo spiral (Dentsply/Maillefer, Ballaigues, Switzerland) and then condensed with finger pluggers (Dentsply/Maillefer). For each specimen new sterile instruments were used. For Groups 2 and 3 the tubes were filled flush with two commercially available non-setting calcium hydroxide products, Calasept (Nordiska Dental, Angelholm, Sweden) and Ultracal XS (Ultradent Products Inc., South Jordan, UT, USA). For these groups the materials were injected using a 30-gauge needle according to the manufacturer's instructions. After removal of excess calcium hydroxide with sterile tissue paper, the specimens were placed in individual stoppered vials, measuring 34 mm in length with an internal diameter of 8.0 mm, each containing 2.5 ml distilled water at pH 7.4, which served as a baseline pH measurement. The tubes were positioned in the center of the vials and extended 15.0 mm into the testing medium (Fig. 1). The vials were also cleaned with 1% hydrochloric acid, rinsed with distilled water and then autoclaved. Five unfilled glass tubes ($n = 5$) were used as negative controls. They were submerged in the medium and positioned into their vials in a fashion similar as described for the experimental groups. In all groups the changes in pH in the medium were measured at 1 and 24 h and 15 and 30 days. Measurements were carried out with a micro-pH electrode and a digital pH meter (Model PH-207, Phoenix Electrode Co, Houston, TX, USA). The pH meter was calibrated with a solution of a known pH, before and after measurements of each time period. In order to standardize the pH readings for all samples, the electrode remained submerged in the medium for 30 s. After completion of the measurements, the microelectrode was rinsed with distilled water and dried with sterile tissue paper to ensure that no calcium hydroxide particles were left behind, as this potentially could interfere with the next reading. Each sample was tested three times and the mean value for each sample of each group calculated. The mean for each experimental group and for the controls was calculated from the means of the 20 samples and the five samples respectively. Before each reading, the medium was homogenized by shaking the vial to ensure uniform distribution of the hydroxyl ions. Between readings the vials were kept closed using the rubber stoppers and stored in an incubator at 37°C. After completion of the measurements, the tubes containing the test materials were immediately returned to their vials and stored until the next measurement. Because of the technique employed, the medium in each vial was reduced with every measurement. At the conclusion of the each session the volume was therefore replenished to the original amount of 2.5 ml by adding new medium with a pH that was equal to the last recorded mean value. The mean pH values and standard deviations for each group were calculated and further subjected to an analysis for which a SPSS 10.0 software (Statistical Package for the Social Sciences Inc., Chicago, IL, USA) was used. An ANOVA repeated measures and two-way ANOVA were used to determine the difference between groups at each time interval. ANOVA and two-way ANOVA were also used to evaluate the significance of the change in pH within each group as a function of time. A significance level was set at $P < 0.05$.

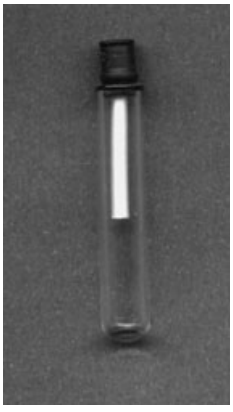

Fig. 1. Photograph of the experimental set-up imitating a periapical environment and showing a 17.0-mm-long glass tube positioned inside the glass vial, which contained 2.5 ml distilled water at pH 7.4. The glass tube was filled with the test calcium hydroxide material, which released hydroxyl ions through a 1.0 mm opening into the medium. The subsequent change in pH was then measured over four time periods.

Results

Table 1 lists the mean and standard deviations of the groups. The pH values indicated that the materials tested in Groups 1, 2, and 3 produced a high alkalinity score. The results of the ANOVA established that the pH changes in the test medium were determined by the composition of the mate-

Table 1. Mean pH levels of the medium at four time intervals (initial pH 7.40)

Material	*n*	1 h	24 h	15 days	30 days
CH + distilled water	20	10.93 (0.16)	11.24 (0.23)	11.26 (0.14)	11.27 (0.13)
Calasept	20	9.82 (0.08)	11.23 (0.27)	11.42 (0.08)	11.77 (0.08)
Ultracal XS	20	9.92 (0.09)	11.26 (0.24)	11.44 (0.09)	11.82 (0.06)
Control*	5	7.40 (0.00)	7.40 (0.00)	7.40 (0.01)	7.40 (0.01)

Numbers in parenthesis = standard deviation.
CH, calcium hydroxide.
*Two-way ANOVA showed no significant changes in pH as a function of time.

rials tested and the time interval at which the test was conducted. After 1 h, the pH of the medium for the paste of calcium hydroxide/distilled water, Calasept and Ultracal XS had experienced a rapid increase with the first being statistically significantly higher ($P < 0.05$) than Calasept and Ultracal XS. The alkalinity continued to increase; however, after 24 h there was no statistically significant difference between the three experimental groups. Although the alkalinity continued to increase over the ensuing time, no statistically significant differences were established after 15 days. At the conclusion of the experiment after 30 days, Calasept and Ultracal XS yielded a significantly higher pH ($P < 0.05$) than the calcium hydroxide/distilled water mixture. A comparison of the results within each group at each time interval demonstrated that the pH values for the calcium hydroxide/distilled water paste showed significant differences between 1 and 24 h ($P < 0.05$). However, from thereon no statistically significant differences were demonstrated. In contrast, there were statistically significant differences for Calasept and Ultracal XS between all time intervals. The pH of the medium of the control group did not show perceptible changes over the 30-day observation period.

All experimental groups had a statistically significantly higher pH compared to the control group ($P < 0.05$).

Discussion

The importance of the alkalinizing effects of calcium hydroxide products used as intracanal dressings and their capacity to produce hydroxyl ions in the periapical environment has been extensively reported (5, 12, 13, 18, 19). It has been demonstrated that the action of inflammatory and clastic cells is enhanced by an acidic pH, leading to disintegration and subsequent resorption of hard tissues (11).

The action of calcium hydroxide, that is the hydroxyl ions, is through the dentinal tubules and the apical foramen, with the latter being the most effective (12). The alkalinity of the periapical tissues that can be achieved by the use of calcium hydroxide compounds and the method of diffusion of hydroxyl ions through the apical foramen is therefore worthy of investigation.

In the present *in vitro* experiment, four observations were made over a 30-day period to determine the release of hydroxyl ions from three non-setting calcium hydroxide preparations into a testing medium in a simulated periapical environment. The design of the study attempted to mimic a clinical situation; however, glass tubes containing the calcium hydroxide compounds were used instead. As it was the intention to only measure pH changes in the medium surrounding the specimens, no pH changes of the materials in the tubes were recorded. The quantity of the medium in the vials was determined based on the results of a pilot study, which demonstrated that the amount used in this study was sufficient for measuring pH changes. This experimental model provided a simple method for reproducible measurements of pH changes of the medium, thus allowing a comparison between different calcium hydroxide materials. The pH values recorded in this experiment were higher than what has been reported by Simon et al. (13). They placed different calcium hydroxide materials in prepared root canals of extracted human teeth. With an inner diameter of the glass tubes of 1.0 mm, the experimental design closely resembled a clinical situation of immature teeth or cases with apical root resorption. Therefore, it was no surprise that the diffusion of hydroxyl ions through this fairly large opening was much higher than for mature teeth (13) in which the diameter of the prepared apical area was much smaller. Another factor that may have affected the results is that in natural human teeth the buffering effect of dentin plays an important role (14). When using glass tubes this issue is entirely non-existent.

The results of Table 1 demonstrated that the paste of calcium hydroxide/distilled water as well as Calasept and Ultracal XS had good alkalinizing properties that affected the surrounding medium. This suggests that hydroxyl ions were able to diffuse into the medium from the material inside the tubes. The fast alkalinizing effects of these materials after 1 and 24 h was because of the immediate contact of the calcium hydroxide with

the medium, resulting in an instantaneous release of hydroxyl ions. The paste of calcium hydroxide/distilled water released hydroxyl ions more rapidly over a 1 h time period, whereas after 24 h no difference with Calasept and Ultracal XS was observed. The faster release of hydroxyl ions within the 1-h period may be an inherent property of calcium hydroxide powder/distilled water formulations (13) or it may be because of the much higher percentage of calcium hydroxide (powder/water weight percent ratio of 50%). For instance, Calasept is composed of calcium hydroxide (41.07%), barium sulfate (8.33%) and other ingredients in a sterile isotonic saline solution. A precise formulation of Ultracal XS is not available but it contains calcium hydroxide (35.0%) and a contrast-enhancing material in a saturated aqueous solution. One may speculate that the slower pH increase from Calasept and Ultracal XS was caused by a reduced availability of calcium hydroxide because of the presence of additional components in these formulations, until over time a saturation level had been reached. After 15 days, the alkalinizing effects of Calasept and Ultracal XS increased gradually, owing to a slow but steady release of hydroxyl ions, whereas the calcium hydroxide/distilled water mixture appeared to have reached its maximum output. At the end of the experiment after 30 days, Ultracal XS showed the highest alkalinity; however, no statistical significance could be demonstrated between Calasept and Ultracal XS, whereas both these materials had reached a statistically significant higher pH than the calcium hydroxide/distilled water mixture. The alkalinizing effects recorded in this experiment need to be viewed in the context of other published research. The vials containing the test materials were kept tightly sealed with rubber caps and only during the pH measurements were the materials exposed to aerobic conditions. According to Fuss et al. (15), the efficacy of calcium hydroxide compounds may be affected by chemical alteration when exposed to ambient air that contains CO_2, which in contact with the aqueous medium will produce an insoluble compound calcium carbonate. They reported a significant reduction in pH levels after carbonation of calcium hydroxide-based materials (15). On the contrary, Cohen & Lasfargues (16) suggested that the reduction in pH levels did not appear to be directly related to the carbonation phenomenon. Their observations were corroborated by a study of Duarte et al. (17). The difference in results between the current study and the one reported by Fuss et al. (15) may be in the experimental design. It seems that the possible influence of carbonation on pH levels induced by calcium hydroxide-based materials needs to be more extensively analyzed. In a clinical situation, CO_2 may also originate from metabolism of microorganisms within the canal or dentinal tubules and from the surrounding tissues (18), a phenomenon that cannot occur under the experimental conditions of this study.

The sustained alkalinizing effects of the tested materials are of clinical significance. Nerwich et al. (19) measured pH changes in root dentin over a 4-week period and considered this a reasonable time interval to expect effective therapeutic benefits from calcium hydroxide-based materials. At the end of the experiment reported here, the pH level of the testing medium was 11.27, 11.77, and 11.82, respectively, for the calcium hydroxide/distilled water mixture, Calasept, and Ultracal XS. At these pH levels, most bacteria cannot grow (19, 20). In an *in vitro* experiment (21), it was shown that a pH > 7.8 may interfere with vital cell functions of human fibroblasts which are necessary for periodontal tissue healing. However, one must keep in mind that the conditions of cell cultures *in vitro*, in particular when cells are in direct contact with calcium hydroxide, do not adequately reflect the *in vivo* situation. In a clinical case, the root canal has to be filled with calcium hydroxide and the presence of a high pH in periapical tissues for a prolonged period of time is unlikely to occur, because of the buffering effect of dentin (14, 22) and/or the dilution effect of connective tissue serum. Additional buffering may also be expected from the high solubility of calcium hydroxide preparations (23, 24) as well as from the acidification process by inflammatory cells (11). Furthermore, calcium hydroxide dressings used in pulp capping procedures, with the intent to stimulate, among other cells, fibroblasts, have clearly established that a pH of 9–11 creates the ideal environment to activate these cells to initiate a repair process (25). Under the conditions of this study, all tested materials with the exception of the unfilled tubes in the control group exhibited a prolonged alkaline effect on the surrounding medium with Ultracal XS generating the highest pH, albeit without statistical significance when compared to Calasept.

Within the limitations of this experimental design, definitive conclusions cannot be drawn and further *in vivo* investigations are needed to establish a correlation between the incremental increase and duration in pH and the biological response of the periapical tissues.

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