Determination of pH and calcium ion release provided by pure and calcium hydroxide-containing AHPlus

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Abstract

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Aim To compare *in vitro* the pH and calcium ion release provided by pure and calcium hydroxide-containing AHPlus.

Method Pure and modified AHPlus, the latter containing 5 and 10% (w/w) calcium hydroxide added during spatulation, were used. The material was spatulated and stored in 10 tubes that were 1 cm long and 1.5 mm in diameter, and then immersed in 20 mL deionized water before the materials had set. Ten tubes with zinc oxide and eugenol were used as controls. Four millilitres of water was removed from the flasks after 24 and 48 h, and after 7, 14 and 30 days, and pH and calcium release were measured with a pH meter and by atomic absorption spectrophotometry, respectively. The results obtained at each time point were compared statistically.

Results A more alkaline pH for AHPlus supplemented with 5 and 10% calcium hydroxide was recorded when compared to pure AHPlus; there were significant differences at 14 and 30 days ($P < 0.05$). The results of calcium ion release showed no significant difference between pure AHPlus and zinc oxide plus eugenol ($P > 0.05$). The comparisons between the AHPlus containing 10% calcium hydroxide with AHPlus containing 5% calcium hydroxide, pure AHPlus, zinc oxide plus eugenol demonstrated significant differences ($P < 0,05$) at all periods. The comparisons between AHPlus containing 5% calcium hydroxide with pure AHPlus and zinc oxide plus eugenol demonstrated significant differences ($P < 0,05$) at all periods of evaluation.

Conclusions The addition of 5 and 10% calcium hydroxide to AHPlus cement favoured a more alkaline pH and greater calcium ion release.

Keywords: AHPlus, calcium hydroxide, pH, root canal obturation, sealer.

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Introduction

The search continues for an endodontic sealers that fulfils the ideal physicochemical properties of sealing, radiopacity, setting time and flow, as well as biological properties. One concern has been to develop materials that exert a stimulating effect on the repair process, i.e. materials that have a biological role in the healing of periapical disease.

Calcium hydroxide has been added to sealing cements and gutta-percha points because of its biological effects. Calcium hydroxide acts at the tissue level by favouring alkaline pH and calcium release, leading to biochemical effects that culminate in the acceleration of the repair process (Seux *et al.* 1991, Estrela *et al.* 1994, 1995).

In order to stimulate mineralization, the material should also release calcium, which reacts with tissue carbonic gas forming calcium carbonate that favours the mineralization (Seux *et al.* 1991).

Berbert (1978) added calcium hydroxide to AH26 and observed an improvement in the biological behaviour of the material in the teeth of dogs. de Moraes (1984), studying the physical characteristics of AH26 cement and AH26 modified by the addition of calcium hydroxide, observed an improvement especially in terms of apical sealing.

One of the first commercially available endodontic sealer containing calcium hydroxide was Sealapex (Sybron

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Kerr Co., Romulus, MI, USA). Holland & de Souza (1985) reported excellent biological results, demonstrating the positive effect of the addition of calcium hydroxide to root canal sealers.

AHPlus (Dentsply DeTrey, Konstanz, Germany) has become available recently with calcium tungstate in its composition, rather then calcium hydroxide.

Duarte & Moraes (2000) have shown that the addition of calcium hydroxide leads to an improved sealing capacity of the material, as determined by methylene blue infiltration. No interference of calcium hydroxide with radiopacity or setting time was observed (Duarte 1999).

The objective of the present study was to determine whether the addition of 5 and 10% calcium hydroxide to AHPlus during spatulation favours an alkaline pH and calcium release from the sealer.

Materials and methods

AHPlus (Dentsply DeTrey) of the following composition was used in the present study:

1 epoxy paste: diepoxy, calcium tungstate, zirconium oxide, aerosol and dye; and

2 amine paste: 1-adamantane amine, *N,N′*-dibenzyl-5-oxanonandiamine-1,9, tricyclo decane (TCD)-diamine, calcium tungstate, zirconium oxide, aerosol and silicon oil.

Based on this sealer, two other experimental sealers were prepared by adding proportionally 5 and 10% (w/w) calcium hydroxide p.a. (Merck, Rio de Janeiro, RJ, Brazil) during spatulation. Zinc oxide and eugenol cement (proportion of 1.8 g mL^{-1}) was used as the negative control.

Determination of pH and calcium ion release

For the determination of pH and calcium release, 40 tubes (1 cm long, 1.5 mm in diameter) were filled with one or other of the materials and immersed immediately in individual glass flasks containing 20 mL deionized water, before the materials had set. The flasks were hermetically sealed and kept in an incubator at 37 °C. Four millilitres of water were removed after 24 and 48 h, and 7, 14 and 30 days for the determination of pH and calcium release. Ten samples were prepared for each group.

The pH was determined with a pH meter (Corning Incorporated, New York, USA). Care was always taken to verify the precision of the apparatus by performing constant measurements using known buffers at pH 4, 7 and 9.

Ca^{2+} release was monitored using an atomic absorption spectrophotometer (Varion Co., São Paulo, Brazil) equipped with a calcium-specific hollow cathode lamp. The operational conditions were: light current, 3 mA; fuel, acetylene; support, oxygen; and stoichiometry, reductor.

Pilot tests were carried out for the correct determination of the wavelength and slit. To prevent possible interferences from phosphate and alkaline metals, the samples and standards were diluted in 10% EDTA, and the glassware was previously washed with nitric acid.

A 10-mg dL^{-1} standard calcium stock solution was diluted in 10% EDTA to obtain the following concentrations: 0.025, 0.05, 0.1, 0.2 and 0.5 mg dL^{-1}, to verify the atomic absorption spectrophotometer's calibration. The samples were diluted as necessary. The apparatus was calibrated at zero absorbance using 10% EDTA as blank. Calcium release was calculated using the line equation of the standard curve and was determined at the same time-points as the pH.

The data were analysed statistically using one-way analysis of variance for the comparison of the materials at each time-point. If the difference was significant, individual comparisons were performed by the Tukey–Kramer test.

Results

Table 1 shows the mean pH and SD as a function of time. Statistical analysis revealed a significant difference ($P < 0.05$) between pure and modified AHPlus and zinc oxide plus eugenol at 24 and 48 h and at 7 days. At 14

Table 1 Mean pH and SD obtained for the cements studied after different periods of observation

	AHPlus 10% $Ca(OH)_2$		AHPlus 5% $Ca(OH)_2$		Pure AHPlus		ZOE	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24 h	7.23	0.10	7.18	0.13	7.10	0.18	6.89	0.10
48 h	7.36	0.17	7.28	0.09	7.21	0.17	7.01	0.03
7 days	7.51	0.14	7.41	0.11	7.38	0.09	6.97	0.05
14 days	7.98	0.03	7.94	0.03	7.43	0.08	7.15	0.02
30 days	8.02	0.04	7.99	0.03	7.76	0.08	7.10	0.04

Table 2 Mean calcium release (mg dL^{-1}) and SD obtained for the cements studied after different periods of observation

	AHPlus 10% $Ca(OH)_2$		AHPlus 5% $Ca(OH)_2$		Pure AHPlus		ZOE	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24 h	0.16	0.01	0.13	0.02	0.10	0.01	0.09	0.005
48 h	0.18	0.01	0.13	0.02	0.10	0.008	0.09	0
7 days	0.21	0.01	0.14	0.01	0.10	0.007	0.09	0.003
14 days	0.39	0.02	0.26	0.01	0.11	0.01	0.10	0
30 days	0.46	0.02	0.35	0.03	0.11	0.01	0.10	0

and 30 days, no significant difference ($P > 0.05$) was observed between AHPlus containing 5 and 10% calcium hydroxide, while the other comparisons showed significant differences ($P < 0.05$).

Table 2 shows the mean calcium release and SD provided by the cements. No significant difference ($P > 0.05$) was observed between pure AHPlus and zinc oxide plus eugenol, while the comparisons between the AHPlus containing 10% calcium hydroxide and that containing 5% calcium hydroxide, AHPlus containing 10% calcium hydroxide and pure AHPlus, AHPlus containing 10% calcium hydroxide and zinc oxide plus eugenol, AHPlus containing 5% calcium hydroxide and pure AHPlus, and AHPlus containing 5% calcium hydroxide and zinc oxide plus eugenol were significantly different ($P < 0.05$) at all periods of evaluation.

Discussion

The methodology used in the present study consisted of placing the material in standard tubes and immersing them in solution prior to determining the pH of the solution with a pH meter (Anthony *et al*. 1982) or potentiometer (Tamburic *et al*. 1993). Some authors have placed the material inside root canals (Simon *et al*. 1995). However, special care should be taken when employing teeth because of potential differences in terms of the size of the apical foramina. Different results have been obtained when this variable was not considered, since smaller contact areas produce lower pH values when the same amount of solution is used to immerse the specimens.

Another factor that could influence results is the nature and type of solution. Duarte (1996) and Duarte *et al*. (2000) replaced the solution after each reading, while in the present study, the tubes were immersed in 20 mL of solution, with 4 mL of the solution being removed at each time-point for pH determination. This latter technique always led to an increase in pH, while replacing the solution could more frequently lead to oscillations.

The flask solution and specimen set was incubated at 37 °C under aerobic conditions, as Fuss *et al*. (1996) did not observe any difference in pH between aerobic and anaerobic conditions.

Various methods have been proposed for the determination of calcium ion release. Estrela & Pesce (1996) studied *in vivo* the release of calcium ions from calcium hydroxide pastes using different vehicles. They used tubes containing the material embedded in dog subcutaneous tissue and removed the tubes after various periods of time and determined the amount of calcium released in the tissue. This method most closely resembles the situation *in vivo*, but its execution is difficult and requires the knowledge of how much calcium the test material contains.

Determination of pH and calcium release showed that the addition of 5 and 10% calcium hydroxide favoured a more alkaline pH and a greater calcium release compared to pure AHPlus cement. This has been reported previously by Tagger *et al*. (1988), da Silva *et al*. (1993) and Duarte *et al*. (2000) who observed that calcium oxide- and calcium hydroxide-containing cements favour an alkaline pH and calcium release. This higher alkalinity and enhanced calcium release should lead to improved biological and microbiological behaviour, as a more alkaline pH favours the deposition of mineralized tissue and exerts an antimicrobial action (Estrela *et al*. 1994, 1995). Calcium favours mineralization as it reacts with tissue carbon gas forming calcium carbonate, a crystal that serves as a nucleus for calcification (Seux *et al*. 1991).

Although pure AHPlus contains calcium tungstate, calcium release was absent from this material, as it had values similar to those obtained for zinc oxide plus eugenol, which were essentially zero. Although the addition of 10% calcium hydroxide favours a more alkaline pH and greater calcium release, the material in that form is thick and has poor flow (Duarte 1999). The addition of 5% calcium hydroxide led to a less viscosity material, as well as provided a more alkaline pH and calcium release; this proportion is preferred.

Conclusions

Based on the method and conditions employed in the present study, it is concluded that the addition of calcium hydroxide to AHPlus favours a more alkaline pH of the material and a significantly greater release of calcium ions.

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