Dental Traumatology 2006; doi: 10.1111/j.1600-9657.2005.00326.x

DENTAL TRAUMATOLOGY

Vehicle influence on calcium hydroxide pastes diffusion in human and bovine teeth

Camargo CHR, Bernardineli N, Valera MC, de Carvalho CAT, de Oliveira LD, Menezes MM, Afonso SE, Mancini MNG. Vehicle influence on calcium hydroxide pastes diffusion in human and bovine teeth. © Blackwell Munksgaard, 2006.

Abstract – The purpose of this study was to compare the pH and calcium ion liberation after use of calcium hydroxide pastes with different paste vehicles in human or bovine teeth. Ninety-two single-rooted human and bovine roots were used. The roots were instrumented and an external cavity preparation was performed. The roots were divided in to human and bovine groups. Each group was subdivided into four subgroups (SB) according to the vehicle: SB1, detergent; SB2, saline; SB3, polyethylenoglycol + camphorated paramonochlorophenol (Calen PMCC) and SB4, polyethylenoglycol + furacyn paramonochlorophenol (FPMC). Specimens were immersed into saline solution at 37°C and after 7 and 14 days pH and calcium ion measurements were made. The results were analyzed by ANOVA and Tukey tests ($P < 0.05$). There was no statistical difference between bovine and human teeth in the pH analysis ($P < 0.05$), but bovine teeth provided larger calcium ion liberation than human teeth. Calen PMCC was statistically more effective for pH increase and calcium ion liberation in all analyses, followed by FPMC and saline. Detergent showed the lowest pH alterations and calcium ion liberation. The period of 14 days showed more calcium ionic liberation than the 7-day period.

Carlos Henrique Ribeiro Camargo[1], Norberti Bernardineli[2], Marcia Carneiro Valera[1], Cláudio Antônio Talge de Carvalho[1], Luciane Dias de Oliveira[1], Marcia Maciel Menezes[1], Samira Esteves Afonso[1], Maria Nadir Gasparoto Mancini[1]

[1]Dental School of São José dos Campos, São Paulo State University, UNESP, Brazil; [2]Dental School of Bauru, São Paulo University, USP, Brazil

Key words: calcium hydroxide; dentin permeability; intracanal dressing

Carlos Henrique Ribeiro Camargo DDS, PhD, Dental School of São José dos Campos, São Paulo State University, UNESP, Brazil.
E-mail: henrique@fosjc.unesp.br

Accepted 28 September, 2004

In several types of dental trauma, the use of calcium hydroxide as intracanal medication is necessary for the success of root canal treatment in different clinical situations, such as: (a) infection of root canal in cases of pulp necrosis after trauma (1–3); (b) necessity of complementally action in the dissolution of organic tissue after the use of chlorine compounds, in atypical root canals, internal resorptions and incomplete, formation (4, 5); (c) repair induction for the formation of mineralized tissues; (d) control of inflammatory and substitutive root resorptions. For this reason calcium hydroxide has been the most utilized intracanal dressing (1), and has been considered the best choice due to its anti-microbial properties, including the increase of pH in the environment (1), action against bacterial lipopolissacaride (2) and CO_2absorption (3) as well as anti-inflammatory action and repair induction (4–6).

To use calcium hydroxide as a paste a vehicle is necessary. This vehicle will determine the speed of ion dissociation, establishing the solubilization capacity. Many studies have evaluated the dentin pH for the hydroxyl ion diffusion through the dentin tubules and the reactions between dentin and calcium hydroxide pastes (3, 7–19). Several studies show that at least 14 days are necessary for the calcium and hydroxyl ions have an effective penetration in the dentin and reach the external surface of the root (6, 10, 12, 14, 15, 18).

There are many vehicles that can be used to make calcium hydroxide pastes such as: saline solution and distilled water (aqueous vehicles), propylenoglicol and polyethylenoglycol (viscous vehicles), that influence in the effects of the medication. To prove scientifically the advantages and disadvantages of different vehicles in the dissociation of calcium and hydroxyl ions is necessary to make possible a better use of this medicament. However, for ethical reasons and considering the great shortage of extracted human teeth, due to preventive treatments and the progress of the dentistry in the preservation of the teeth, we have had some difficulties to obtain human teeth, therefore the use of alternative models is necessary. Bovine teeth can be a viable alternative for the standardization of researches involving dentin permeability (20). In this way, it is hoped that the bovine teeth present similar conditions to the human teeth in the diffusion of calcium and hydroxyl ions. The study of different vehicles in calcium hydroxide diffusion into dentin and root canal system of human and bovine teeth is extremely important to make possible more studies to help the clinical use of the pastes. The purpose of this study was to compare the diffusion of calcium hydroxide pastes with different vehicles, by evaluating pH alterations on external root dentin and calcium ion liberation, determining which vehicle had better results and if bovine and humans teeth had the same behavior in this study.

Materials and methods

Ninety-two extracted teeth were used in this study, being 46 humans premolars extracted for orthodontics reasons and 46 bovine incisors. All teeth were cleaned and stored in 10% formaldehyde solution for 24 h and after in saline solution until the use. The teeth had the crowns sectioned, the apical foramen of each root was enlarged up to a size No. 30 K-file and the work length was determined 1 mm from the apex. The canals were instrumented at the work length up to a size No. 40 K-file in human' teeth and up to a size No. 80 K-file in bovine teeth. This difference in instrumentation was due to anatomic size variations between bovine and human teeth. The canals were then prepared by step back technique using Gates Glidden drills Nos 1 and 2 for human teeth and Nos 3 and 4 to bovine teeth (Maillefer – Switzerland), and the cervical third of the canals were regularized with a 2082 diamond bur (KG Sorensen) in high speed to standardize the specimens by the diamond bur diameter. During all preparation, the canals were irrigated with 2.5% sodium hypochlorite (Therapeutics – Drugstore LTDA – São José dos Campos SP – Brazil) and a final flush with 17% EDTA (Therapeutics – Drugstore LTDA – São José dos Campos SP – Brazil) for 3 min that was rinsed with saline solution. The roots were placed in a flask containing saline solution for 14 days.

In the middle third of buccal surface of each specimen, a rectangular cavity was prepared using a modified microscope coupled with a precision micrometer that positioned the root and the high-speed hand piece (Fig. 1). The cavities were standardized using a No. 2094 diamond bur to 4 mm of length, 2 mm of width and 1 mm of depth. With X-rays performed before and after the

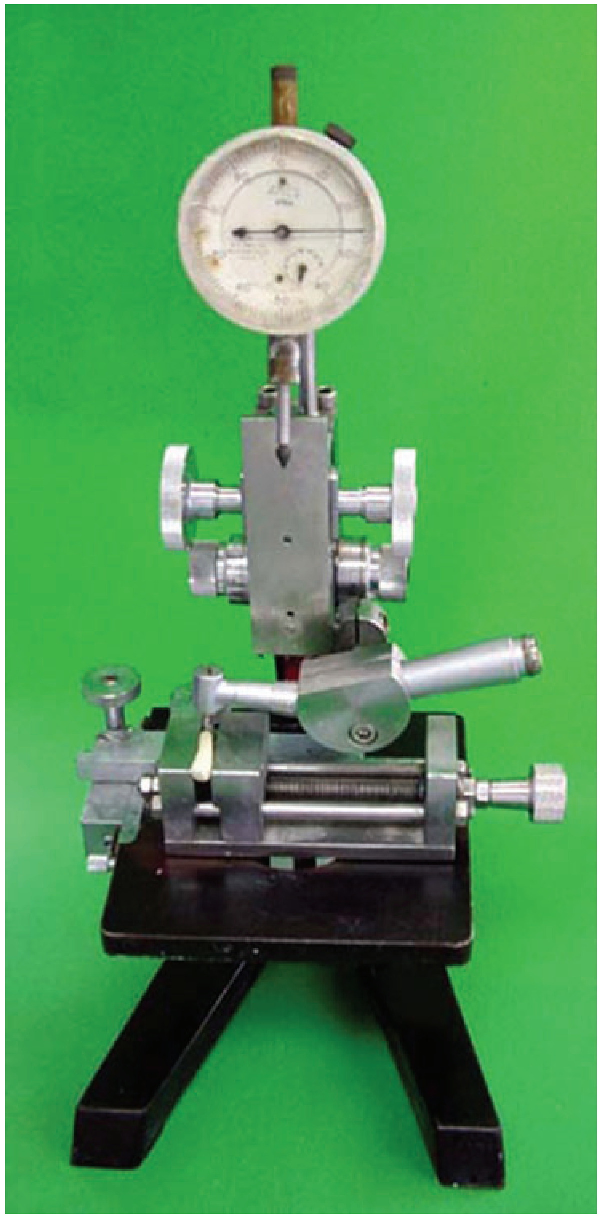

Fig. 1. Modified microscope coupled with a precision micrometer that positioned the root and the high speed.

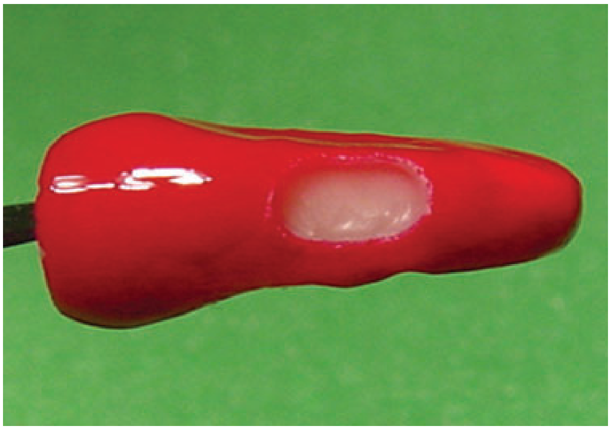

Fig. 2. The standardization cavity, with 4 mm of length, 2 mm of width and 1 mm of depth.

accomplishment of the cavities, it was possible to leave for all roots the same amount of dentin between the cavity floor and root canal wall (Fig. 2).

After root canal preparation, the human and bovine roots were filled with calcium hydroxide pastes, and divided into four subgroups of 20 teeth per group (10 humans and 10 bovines) according to the vehicle used:

Subgroup 1 – calcium hydroxide powder (Therapeutics Drugstore – São José dos Campos SP – Brazil) associated to a detergent (aqueous vehicle – Tergestesin – Degussa – Brazil) in the proportion of 1 g for 1 ml.

Subgroup 2 – calcium hydroxide powder with saline solution (aqueous vehicle) in the proportion of 1 g of calcium hydroxide for 1 ml of saline solution.

Subgroup 3 – calcium hydroxide powder, in viscous vehicle, polyethylenoglycol 400 + camphorated paramonochlorophenol (paramonochlorophenol PMC, 25% for 75% of camphor; Calen PMCC, SSWhite, Brazil).

Subgroup 4 – calcium hydroxide powder in viscous vehicle, polyethylenoglycol 400 + furacyn paramonochlorophenol (FPMC) (5 g of paramonochlorophenol for 28 ml of Furacyn Oto-Solution) in the proportion of 1 g of calcium hydroxide for 0.36 ml of FPMC and 0.64 ml of polyethylenoglycol 400 (Terapêutica – Drugstore – São José dos Campos – SP – Brazil).

Calcium hydroxide pastes were placed into the root canals by lentulo drills (Maillefer –Switzerland) until apical extrusion. The coronal opening and the external surface of the roots were coated with a nail polish layer and a layer of sticky wax, except in the cavity area. Each specimen was immersed in 4 ml of sterile saline solution in individual plastic flasks that were previously washed in 25% hydrochloric acid. After, the specimens were rinsed in tap water and stored at 37°C.

Two human premolars and two bovine incisors were used as positive control 1 (CP1), that were filled with calcium hydroxide manipulated with saline solution and did not receive external coating. Two other human premolars and two bovine incisor were used as positive control 2 (CP2), that were not filled out with calcium hydroxide and received coating, except in the cavity preparation area and, other two human premolars and two bovine incisor were used as negative controls (CN), receiving intracanal dressing with calcium hydroxide manipulated with saline solution and totally coated with nail varnish and wax.

The pH measurements were performed after 7 and 14 days using a precision pHmeter (Hanna model HI 9224), coupled to a conventional electrode (Mettler Toledo – Brazil), that was placed in the saline solution in which each specimen was submerged.

To verify calcium ion liberation, 0.04 ml of saline solution were removed from each flask and placed in tubes containing 2 ml of a calcium ion mark solution (Labtest Diagnóstica – São Paulo SP – Brazil) that changed the colour solution when calcium ions were present. The verification of calcium ion liberation was performed twice for each specimen. The solutions were then transferred to a vial to read the calcium ion liberation using a UV spectrophotometer (Shimatzu 1203) with a wavelength of 570 nm. The data obtained with the spectrophotometer was established in absorbance and transformed in milligrams of calcium per deciliters of saline solution. The pH and calcium ion liberation data obtained were recorded and the results were analyzed and submitted to the statistical analysis (ANOVA 2) and Tukey test at the significance level of 5%.

Results

The means of pH measurements and calcium ion liberation for human and bovine specimens are expressed in Tables 1 and 2.

The ANOVA and Tukey tests showed differences between the groups. There was no statistical difference between bovine and human teeth in the pH analysis in all subgroups, but bovine teeth provided larger calcium ion liberation than human teeth, with statistical difference ($P = 0.0001$). Calen PMCC showed better results for pH increase and calcium ion liberation, statistically different than saline and detergent vehicles ($P < 0.05$); furacyn was not statistically different from Calen PMCC. Furacyn showed larger pH increase and calcium ion liberation than saline and detergent, with statistical difference from detergent ($P = 0.0001$). The period of 14 days showed more ionic liberation with statistical difference from 7 days, except for furacyn pH analyses that showed decrease of pH after 14 days.

Discussion

The high pH promoted by intracanal calcium hydroxide pastes is important to the success of endodontic treatment, especially for treatment of teeth with necrotic pulps. Haapasalo et al. (13) and Çalt et al. (9) showed that calcium hydroxide dressing can be inactivated by dentin because of its buffering capabilities. However, in the present study, it was verified that calcium hydroxide diffused in dentin promoting high pH values, as verified by other studies (3, 7, 8, 10, 14, 15, 18).

Evaluating Tables 1 and 2, it is verified that in both human and bovine teeth the pH means and calcium ion liberation were larger when CPMC was associated to the polyethylenoglycol 400 (Calen PMCC). The differences were statistically significant when subgroup 3 was compared to the subgroup in that detergent (SB1) and saline solution (SB2) were used. These results are in agreement with Anthony et al. (8), that compared the pH values of three vehicles associated to calcium hydroxide (cresatine, saline solution and CPMC), verifying larger values for CPMC, probably because the formation of calcium paraclorofenolate salt that promotes a sustained release and a stable action of $Ca(OH)_2$. Simon et al. (18), studied the effects in the pH and calcium ion liberation of four vehicles associated to calcium hydroxide (distilled water, saline solution, CPMC and propylenoglicol), also verifying larger values for CPMC. Esberard et al. (10), compared pH alterations in different $Ca(OH)_2$ dressings (calcium hydroxide + CPMC and calcium hydroxide + aqueous vehicle − Pulpodent) finding better results for CPMC. Fuss et al. (3) verified larger pH values for aqueous vehicles, in relation to CPMC, but without statistical differences and Alaçam et al. (7) found similar results for pH alterations using $Ca(OH)_2$ pastes in distilled water and glycerin after 12 days.

Calen PMCC's (calcium hydroxide + camphorated paramonochlorophenol) best results can be explained by the better flow of viscous vehicles, resulting in a better contact of the paste with dentin walls, promoting a better filling of the root canal and consequently larger ionic liberation (10). Besides, in the present study, it could be observed that aqueous vehicles, mainly saline solution, evaporated faster than the viscous vehicles, probably due to the smallest superficial tension of aqueous vehicles. Such fact was verified by Hosoya et al. (14), in which the increase of water in the paste took to a larger ionic liberation. Özcelik et al. (16) verified a smaller superficial tension in aqueous vehicles (isotonic saline solution) in relation to viscous ones (glycerin). Safavi and Nakayama (17) studied ionic conductivity of different vehicles and pointed out that aqueous vehicles provided larger conductivity in relation to viscous vehicles.

Group 4 (FPMC) should present better results than Calen PMCC because furacyn is hydrosoluble and the camphor is not. However, higher pH and calcium ion liberation values were verified when Calen PMCC was used, probably because the zinc

Table 1. Means of pH values for human and bovine roots for subgroups 1–4 and controls at 7 and 14 days

	Groups													
	SB1 (Detergent)		SB2 (Saline)		SB3 (Calen PMCC)		SB4 (FPMC)		CP1		CP2		CN	
Period (days)	7	14	7	14	7	14	7	14	7	14	7	14	7	14
Human	7.17	7.86	7.72	7.94	8.53	8.89	8.31	8.05	9.42	11.55	6.63	6.79	7.02	7.03
Bovine	7.13	8.00	7.34	8.43	8.28	8.90	8.27	8.15	10.25	11.26	6.70	6.82	6.10	6.15

SB: subgroup; CP: positive control; CN: negative control.
At 0 day the saline solution pH was 7.14.

Table 2. Means of ion calcium (mg dl^{-1}) for human and bovine roots for subgroups 1–4 after 7 and 14 days

	Groups													
	SB1 (Detergent)		SB2 (Saline)		SB3 (Calen PMCC)		SB4 (FPMC)		CP1		CP2		CN	
Period (days)	7	14	7	14	7	14	7	14	7	14	7	14	7	14
Human	6.33	10.32	6.90	10.97	10.29	14.40	7.85	10.77	20.22	20.37	0.8	1.2	0.05	0.08
Bovine	6.75	10.65	8.11	13.26	11.17	15.19	9.68	12.19	20.37	20.39	1.0	1.2	0.07	0.08

SB: subgroup; CP: positive control; CN: negative control.

oxide presence and colophony that makes the pastes properties better (1).

Regarding the period of evaluation (7 and 14 days), in all subgroups except for subgroup 4 (FPMC), the pH values and calcium ion liberation increased from 7th to 14th day, showing that the pastes maintained the ionic dissociation. The pH decrease in subgroup 4 (FPMC) from 7th to 14th day might have happened because the decrease of the hydroxyl ions after the seventh day, this fact is expected in aqueous vehicles due to the fast ionic dissociation.

The comparison between human and bovine teeth was done due to ethical limitations observed in obtaining human teeth for laboratory studies. It was verified that there was no statistical difference for the pH factor between the values obtained in the bovine and human groups. However regarding calcium ion liberation, bovine teeth presented larger ionic liberation than human teeth, but with the same results among the subgroups. This probably occurred due to the largest caliber of dentin tubules of bovine incisors in relation to the human premolars. Schmalz et al. (20), analyzing *in vitro* the dentin permeability characteristics of human and bovine teeth, verified that these types of teeth could be considered similar. The results observed in the present study regarding bovine teeth showed, especially for pH alterations values, that it is possible to use bovine teeth for studies in this area.

The results of the present study indicate that Calen PMCC is the best option of intracanal dressing in cases of necrotic pulp, with permanence period of at least 14 days. We believe that pH and calcium ion liberation would continue increasing for the other groups until the complete saturation. However, more studies should be performed, to clarify the physical properties of calcium hydroxide pastes and to verify the different actions of this intracanal dressing.

References

1. Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifacio KC, Ito IY. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod 2000;26:391–4.
2. Buck RA, Cai J, Eleazer PD, Staat RH, Hurst HE. Detoxification of endotoxin by endodontic irrigants and calcium hydroxide. J Endod 2001;27:325–7.
3. Fuss Z, Rafaeloff R, Tagger M, Szajkis S. Intracanal pH changes of calcium hydroxide pastes exposed to carbon dioxide in vitro. J Endod 1996;22:362–4.
4. Fava LRG, Saunders WP. Calcium hydroxide pastes: classifications and clinical indications. Int Endo J 1999;32:257–82.
5. Foreman PC, Barnes IE. A review of calcium hydroxide. Int Endod J 1990;23:283–97.
6. Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root canal filling with calcium hydroxide. J Endod 1980;7:17–21.
7. Alaçam T, Yoldas O, Gülen O. Dentin penetration of 2 calcium hydroxide combinations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;86:469–72.
8. Anthony DR, Gordon TM, Del Rio CE. The effect of three vehicles on the pH of calcium hydroxide. Oral Surg Oral Med Oral Pathol 1982;54:560–5.
9. Calt S, Serper A, Ozcelik B, Dalat MD. pH changes and calcium ion diffusion from calcium hydroxide dressing materials through root dentin. J Endod 1999;25:329–31.
10. Esberard RM, Carnes DL Jr, Del Rio CE. Changes in pH at the dentin surface in roots obturated with calcium hydroxide pastes. J Endod 1996;22:402–5.
11. Foster KH, Kulild JC, Weller RN. Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. J Endod 1993;19:136–40.
12. Gomes IC, Chevitarese O, de Almeida NS, Salles MR, Gomes GC. Diffusion of calcium through dentin. J Endod 1996;22:590–5.
13. Haapasalo HK, Siren EK, Waltimo TM, Orstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine: an in vitro study. Int Endod J 2000;33:126–31.
14. Hosoya N, Takahashi G, Arai T, Nakamura J. Calcium concentration and pH of the periapical environment after applying calcium hydroxide into root canals in vitro. J Endod 2001;27:343–6.
15. Nerwich A, Figdor D, Messer HH. pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. J Endod 1993;19:302–6.
16. Özcelik B, Tasman F, Ogan C. A comparison of the surface tension of calcium hydroxide mixed with different vehicles. J Endod 2000;26:500–2.
17. Safavi KE, Nakayama TA. Influence of mixing vehicle on dissociation of calcium hydroxide in solution. J Endod 2000;26:649–51.
18. Simon ST, Bhat KS, Francis R. Effect of four vehicles on the pH of calcium hydroxide and release of calcium ion. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;80:459–64.
19. Vongsavan N, Matthews RW, Matthews B. The permeability of human dentine in vitro and in vivo. Arch Oral Biol 2000;45:931–5.
20. Schmalz G, Hiller KA, Nunez LJ, Stoll J, Weis K. Permeability characteristics of bovine and human dentin under different pretreatment conditions. J Endod 2001;27:23–30.