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Effect of calcium hydroxide-based materials on periapical tissue healing and orthodontic root resorption of endodontically treated teeth in dogs

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Abstract – This study evaluated periapical tissue healing and orthodontic root resorption of endodontically treated teeth sealed with calcium hydroxide in dogs. The sample consisted of three contralateral pairs of maxillary incisors and two contralateral pairs of mandibular incisors in each of two dogs using a split mouth design. After biomechanical preparation of the teeth in the first group ($n = 10$), a $Ca(OH)_2$ dressing was placed for 14 days before root canal filling with $Ca(OH)_2$-based sealer (Sealapex) and gutta-percha points. In the second group ($n = 10$), root canals were obturated immediately after the mechanical preparation with gutta-percha points and zinc oxide and eugenol (ZOE)-based sealer (Endofill). After completion of endodontic treatment, the teeth were moved with an orthodontic appliance with a calibrated force of 200 g, reactivated every 21 days. After 105 days, the animals were killed and the teeth were removed upon completion of active treatment, without a period of recovery, and prepared for histomorphological analysis. All sections of each tooth were graded subjectively on a scale from one to four to obtain the average of the 16 histomorphological parameters analysed. Evaluation of the differences between the two treatment protocols was made with Mann–Whitney *U*-test. It was observed that the teeth treated with $Ca(OH)_2$-based materials provided better outcomes ($P = 5\%$), with complete repair of all root resorption areas, high rate of biological closure of the main canal and apical accessory canals by newly formed cementum, less intense and extensive chronic inflammatory infiltrate, and better organization of the periodontal ligament. Under the tested conditions, $Ca(OH)_2$-based materials had a favourable action on periapical tissue healing and repair of orthodontic root resorption in endodontically treated dogs' teeth.

Orthodontic movement of endodontically treated teeth has been extensively investigated (1–6). However, a consensus has not yet been reached regarding the effects of orthodontic movement of these teeth. While some authors have reported that endodontically treated teeth are more susceptible to root resorption during orthodontic treatment (1, 7), others either have found no significant differences (2, 8) or have reported that orthodontically moved root-filled teeth are less susceptible to root resorption (3, 9). These different outcomes may be ascribed to the type of tooth movement (10).

The findings of a recent study (6) on endodontically treated dogs' teeth with experimentally induced periapical lesions followed by orthodontic movement showed a high periapical healing rate without significant differences in the repair of root resorption areas compared with control teeth not moved. The authors attributed these similar outcomes to the fact that $Ca(OH)_2$-based materials were used for root canal therapy in both groups. The beneficial effect of the $Ca(OH)_2$ on dentine has already been demonstrated regarding the resorption process and new cementum formation in surgically prepared root cavities in animals' teeth (11, 12). This effect added to its osteogenic potential (13) and biocompatibility (14) suggests that it may have a favourable effect on root resorption triggered by orthodontic movement.

The purpose of this study was to evaluate the influence of $Ca(OH)_2$-based materials on the healing of periapical tissues and root resorption in endodontically treated dogs' teeth during orthodontic movement.

Materials and methods

Animal and sample preparation

Twenty maxillary and mandibular incisors of two adult dogs were used in compliance with the Ethical Guidelines of Animal Experimentation after approval by the UNESP-Dental School of Araçatuba Animal Care and Use Committee. For all treatment sessions, a combination of xylazine sulphate (Coopazine®; Coopers do Brasil Ltda, São Paulo, SP, Brazil) and tiletamine hydrochloride:zolazepam hydrochloride (Zoletil®-50; Virbac do Brasil, Indústria e Comércio Ltda, São Paulo, SP, Brazil) was used to anaesthetized the animals. Sixty days before the start of the endodontic–orthodontic treatment, the mandibular central incisors were extracted in order to gain space to allow orthodontic movement of the remaining mandibular incisors.

Experimental procedures

The endodontic treatment started with rubber-dam isolation of the selected teeth and antisepsis of the operative field followed by coronal opening and pulpectomy. Root canal preparation was performed according to a crown-down technique limited to the apical cemental barrier up to #40 K-file (Maillefer Instruments SA, Ballaigues, Switzerland). This barrier was penetrated with a #15 K-file and the cemental canal was widened up to #25 K-file. Saline was used as an irrigant at all stages of root canal therapy because of its neutral pH. The instrumented root canals were dried with absorbent paper points.

After completion of the biomechanical preparation, the experimental teeth were treated as follows: group I – root canals of three maxillary and two mandibular incisors on one side in each of two animals ($n = 10$) received initially a $Ca(OH)_2$/distilled water dressing for 14 days before definitive filling with gutta-percha points and a $Ca(OH)_2$-based sealer (Sealapex; Kerr, Romulus, MI, USA) by the active lateral condensation technique; group II – root canals of the contralateral incisors ($n = 10$) were filled in the first section with gutta-percha points and a zinc oxide and eugenol (ZOE)-based sealer (Endofill; Dentsply, Petrópolis, RJ, Brazil) by the active lateral condensation technique. In both groups, the access cavities were restored with a composite resin (Filtek Z-250; 3M/ESPE, St. Paul, MN, USA).

Orthodontic appliances were fixed to the endodontically treated incisors to initiate tooth movement. In the maxillary incisors, the orthodontic procedures were performed independently at the left and right sides, in a similar manner. A conventional orthodontic bracket (Dental Morelli, Sorocaba, SP, Brazil, code 10.30.203) was bonded to the canine and a $0.019' \times 0.025'$ stainless steel archwire (Dental Morelli) was passively adapted to the buccal surface of the incisors and attached to the bracket slot on the canine. A loop was fabricated distally to the lateral incisor and the archwire was fixed to the central, intermediate and lateral incisors with light-cured composite resin. The force was released by chain elastics (3M Unitek, Monrovia, CA, USA) tied to the canine bracket and the loop on the lateral incisor, with a force calibrated to 200 g upon activation. For orthodontic movement of mandibular incisors, brackets were bonded to the intermediate and lateral incisors at each side with a stainless steel archwire passively adapted to the bracket slots on the incisors, as described for the maxillary teeth with a force calibrated to 200 g upon activation. The elastics of both maxillary and mandibular incisors were changed every 21 days.

Specimen processing

At 105 days, the animals were killed by an anaesthetic overdose and the mandibular and maxillary blocks containing the experimental teeth were removed, fixed in buffered neutral 10% formalin, decalcified in 17% ethylenediamine tetraacetic-acid (EDTA) solution and embedded in paraffin. The specimens were sectioned longitudinally in a buccolingual direction at 6 μm thickness and the sections were stained with haematoxylin and eosin and some of them were stained by the Brown and Brenn technique.

Histological evaluation

Tissue response was evaluated under light microscopy by a skilled observer blinded to the treatment groups. The histomorphological parameters used in this study were based on the criteria proposed by Holland et al. (15) (Table 1). The specimens were examined and each parameter was scored 1–4, one being the best result, four being the worst, and two and three representing intermediate values. All sections of each tooth were examined, individual scores were given to the histomorphological parameters and then a final score was obtained to each parameter. The values of the 16 parameters presented on Table 1 correspond to the means of the scores attributed to the 10 teeth of each group.

Statistical analysis

All scores relative to the 16 parameters of all specimens were submitted to statistical analysis. Mann–Whitney U non-parametric test was used to analyse differences for each parameter (Table 1) and for the overall characteristics of the groups. Significance level was set at 5%.

Results

Group I – $Ca(OH)_2$-based materials

The thickness of newly formed cementum ranged from 15 to 150 μm (mean thickness of 47 μm). This newly formed cementum covered the pre-existing cementum in all teeth. Cementum deposition caused biological closure of the root canals at the apical delta in all teeth (Fig. 1). Complete closure of the foramen of the main root canal

Table 1. Mean scores obtained for the two study groups according to the 16 histomorphological parameters analysed

Histomorphological parameters	Group I, $Ca(OH)_2$-based materials (n = 10)	Group II, ZOE-based sealer (n = 10)
Thickness of newly formed cementum	2.10	2.20
Extension of newly formed cementum	1.00[1]	1.50[2]
Biological closure of accessory canals	1.00[1]	2.50[1]
Biological closure of the main canal	1.80[1]	3.80[2]
Cementum resorption	1.10	1.50
Bone tissue resorption	2.40	2.20
Presence of bacteria	1.00	1.00
Intensity of acute inflammation	1.00	1.00
Extension of acute inflammation	1.00	1.00
Intensity of chronic inflammation	1.50[1]	3.80[2]
Extension of chronic inflammation	1.70[1]	3.40[2]
Thickness of periodontal ligament	3.00	3.50
Organization of periodontal ligament	1.70[1]	3.10[2]
Limit of root canal filling	1.00[1]	2.20[2]
Presence of debris	1.40	2.20
Presence of giant cells	1.00	1.00
Overall mean	1.48[1]	2.24[2]

The $Ca(OH_2)$-based materials [1]exhibited significantly better results (P < 0.05) than those observed with the ZOE-based sealer[2].
ZOE, zinc oxide and eugenol.

being observed in five cases (Fig. 2) and partial closure in two. A root resorption was observed only in one tooth, which was completely filled by newly formed cementum (Fig. 3). Areas of bone tissue resorption were observed in eight specimens. A few areas of active resorption were seen (Fig. 3). Mild chronic inflammatory process was observed in five specimens. The thickness of the periodontal ligament ranged from 225 to 600 μm (mean thickness of 392 μm). Organization of the periodontal ligament was partial in seven cases and filled the entire apical portion in three (Fig. 4). There was no overfilling of any teeth. Presence of debris, basically composed of dentin chips, was observed in four specimens. No giant cells or microorganisms were found.

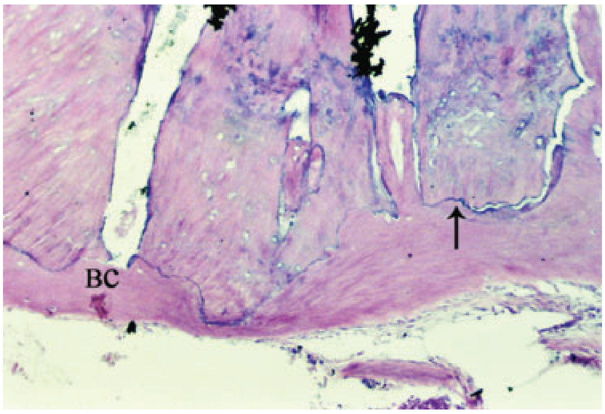

Fig. 1. Group I – $Ca(OH)_2$-based materials. Observe biological closure of canals at the apical delta (BC) and repair of areas of cementum resorption by newly formed cementum (arrow). Haematoxylin and eosin, 100×.

Fig. 2. Group I – $Ca(OH)_2$-based materials. Biological closure of the main canal by newly formed cementum (BC). Presence of few chronic inflammatory cells. Haematoxylin and eosin, 200×.

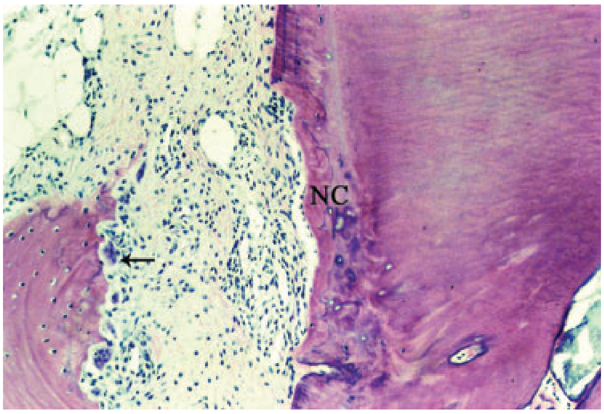

Fig. 3. Group I – $Ca(OH)_2$-based materials. Observe area of root resorption entirely covered by newly formed cementum (NC). There is active resorption of bone tissue (arrow). Haematoxylin and eosin, 100×.

Group II – ZOE-based sealer

Newly formed cementum was observed on all teeth and its thickness ranged from 15 to 37 μm (mean thickness of

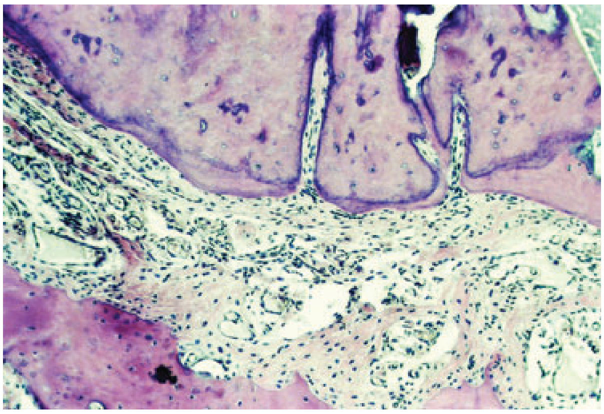

Fig. 4. Group I – $Ca(OH)_2$-based materials. Note vital pulp stump and well-organized periodontal ligament. Haematoxylin and eosin, 100×.

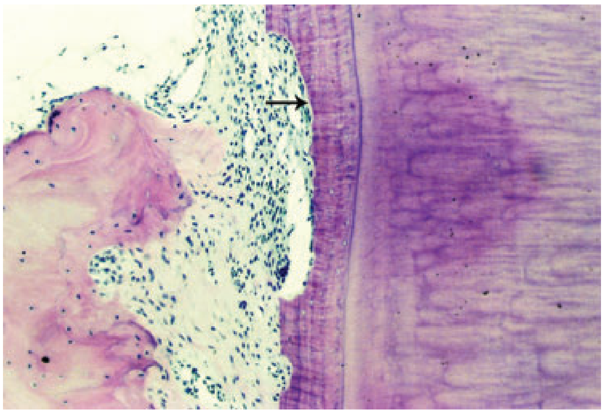

Fig. 5. Group II – ZOE-based sealer. Presence of areas of root resorption affecting only the cementum. Part of these areas is covered by newly formed cementum (arrow). Haematoxylin and eosin, 100×.

25 μm). This cementum covered the pre-existing cementum or partially repaired the areas of root resorption (Figs 5–7). Biological closure of the main foramen was not observed in any case (Fig. 8). However, there was biological closure in some canals at the apical delta, whose remaining pulp tissue was in contact with debris, basically composed of dentin chips and smear layer. Areas of root resorption were observed affecting only the cementum (Fig. 5) or cementum and dentin (Figs 6 and 7). These areas of resorption were partially covered by newly formed cementum. Areas of bone tissue resorption were observed in eight cases, in four with inactive resorption and four with few areas of active resorption. No specimen exhibited acute inflammatory infiltrate. However, all specimens presented lymphoplasmocitary infiltrate, usually with great intensity and extension (Fig. 8). The thickness of the periodontal ligament ranged from 300 to 750 μm (mean thickness of 482 μm). The ligament was disorganized in five specimens and partially organized in the others. The filling material was slightly below the level of the apical foramen in one case (Fig. 8) and at the level of the apical foramen or reaching the periodontal ligament in the others. Presence of debris was observed in seven cases. Giant cells and microorganisms were not observed.

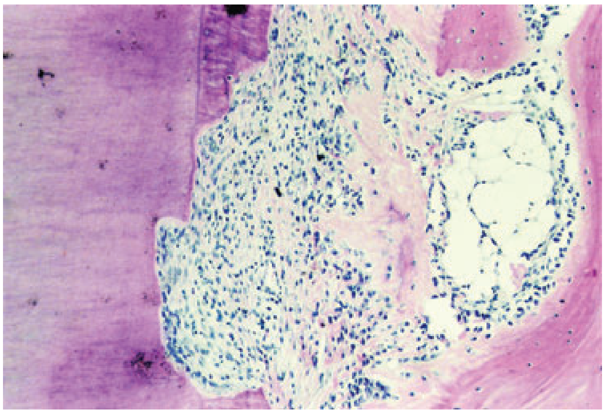

Fig. 6. Group II – ZOE-based sealer. Root resorption affected the cementum and dentin. Observe deposition of a thin layer of newly formed cementum on the resorbed surface. The area of bone resorption is being repaired. Presence of chronic inflammatory infiltrate. Haematoxylin and eosin, 100×.

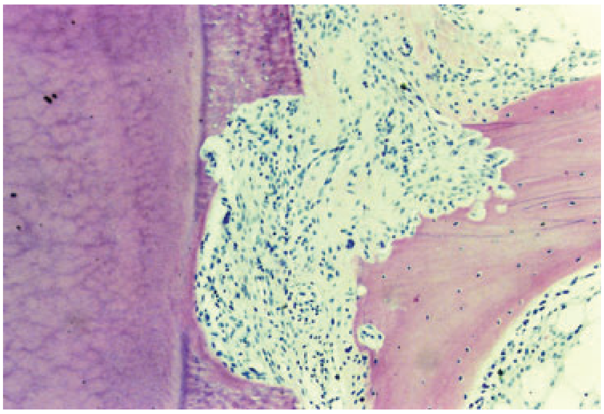

Fig. 7. Group II – ZOE-based sealer. The area of root resorption affected the limit of dentin. Active bone resorption and chronic inflammatory infiltrate in the periodontal ligament. Haematoxylin and eosin, 100×.

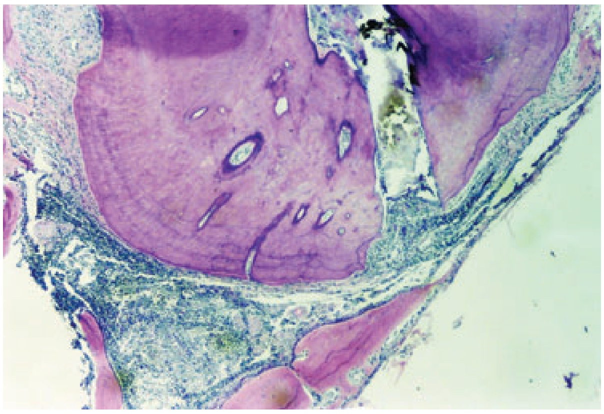

Fig. 8. Group II – ZOE-based sealer. There is intense chronic inflammatory infiltrate in the periodontal ligament. Observe the area of root resorption at the upper left region and right apical region. Haematoxylin and eosin, 40×.

The mean scores of the different histomorphological parameters assigned to all specimens in both experimental groups are presented in Table 1. Statistical analysis revealed the superiority of the group treated with $Ca(OH)_2$-based materials on the group treated with ZOE-based sealer. Two-by-two comparisons revealed statistically significant difference between groups ($P < 0.05$) as to the extension of newly formed cementum, biological closure of the accessory and main root canals, intensity and extension of chronic inflammation, organization of the periodontal ligament and limit of root canal filling.

Discussion

The overall analyses of the 16 histomorphological parameters in this study demonstrated that the group treated with $Ca(OH)_2$-based materials [$Ca(OH)_2$/distilled water dressing and Sealapex sealer] presented significantly better repair than the group treated with ZOE-based sealer (Endofill). This outcome was related to the extension of the newly formed cementum, which repaired the resorption areas, closed the accessory and main root canals, limited the intensity and extension of chronic inflammatory infiltrate and favoured the organization of the periodontal ligament.

Newly formed cementum repairing areas of tooth resorption caused by orthodontic movement has been well demonstrated (6, 16, 17). It has been observed that the peak of resorption occurs between 2 and 4 weeks, is stabilized between 6 and 8 weeks and then decreases after 70 days (18). It has also been demonstrated that the repair of resorption lacunae by new cementum formation occurs even in the presence of forces, initially at the periphery, with concomitant observation of active resorption at the centre (19). Additionally, the findings of a previous study (16) showed that 1 week after completion of orthodontic movement, 28% of resorption lacunae presented some type of repair and at 8 weeks this value increased to 75%. In the present study, after 90 days of orthodontic movement, root resorptions were present in the group of teeth treated with Endofill sealer, sometimes reaching the dentine, which were partially repaired, in agreement with the aforementioned studies. However, in the group treated with calcium hydroxide-based materials, only one case exhibited areas of cementum resorption at the lateral root walls, which were entirely repaired. The presence of areas of bone resorption in five cases demonstrates that orthodontic force was active in the specimens of this group.

These data corroborate the hypothesis suggested by Souza et al. (6), that the use of calcium hydroxide-based materials in endodontic treatment may favourably influence the repair of root resorptions in teeth with chronic periapical lesions before orthodontic movement. This favourable effect on repair of idiopathic external root resorption has been previously mentioned in case reports (20–22) and is assigned to $Ca(OH)_2$-induced pH increase of root structures. It has been demonstrated to occur throughout the dentine within a period of 2–8 weeks (23–25) and in simulated periapical environment (26, 27). The alkaline pH can neutralize the acidic products from clastic cells, preventing dissolution of the mineral component (28). In addition to this neutralization, with possible reduction of root resorption, it has been reported that a high concentration of calcium ions deriving from $Ca(OH)_2$ would activate the alkaline phosphatase, favouring the formation of new calcified tissue (29).

In the present study, the increase in the pH of dentine (23, 24) and periapical region (26, 27) in the group of teeth that received $Ca(OH)_2$-based materials was probably achieved by the placement of a $Ca(OH)_2$ dressing for 14 days and maintained thereafter by the $Ca(OH)_2$ sealer (25, 30). This condition, allied to the 'osteogenic' capacity of $Ca(OH)_2$ (13) could be the main factor responsible for the high rate of complete repair of root resorption and biological closure of the accessory and main root canals. In addition, the biocompatibility of the $Ca(OH)_2$-based material (14, 31) allowed less intense and extensive inflammatory infiltrate and, consequently, better organization of the periodontal ligament in this group.

Regarding to the limit of root canal filling, in most root canals obturated with Endofill sealer, the material always reached or was slightly beyond the level of the apical foramen. This may have occurred because of the higher flowability of ZOE sealers and the moment when obturation was performed. In the group where Sealapex was used, root canals were filled 2 weeks after biomechanical preparation, which was time enough to allow reorganization of the periodontal ligament after over-instrumentation, even with tissue ingrowth into the main cemental canal in some cases. On the other hand, in the group obturated with Endofill sealer, obturation was performed at the first session, when the cemental canal was still empty and the periodontal ligament close to the main foramen was disorganized because of overinstrumentation. This unfavourable condition (15) may influence the periapical tissue healing, but not the root resorption induced by orthodontic movement.

The tooth movements were controlled by tipping and translation with bracket slots of $0.022' \times 0.028'$ and with an $0.019' \times 0.025'$ archwire. There is no consensus in the literature as to the better force that should be applied on teeth for preservation of periodontal structures. A recent study reported that intrusive orthodontic force caused external root resorption in the middle and apical third above 70% of the cases (32). On the other hand, Owman-Moll et al. (10) conducted a clinical–histological study and observed that tooth movement with continuous forces was more effective than tooth movement with interrupted forces, with no damage to the root structure. In the present study, space closure was performed with chain elastics, calibrated at 200 g at movement start. According to Baty et al. (33), chain elastics lose 19.33% of force at 4 h after activation, without further significant changes up to 3 weeks. Thus, the type of force applied in this study may be considered mild and continuous.

The present results indicate that additional studies should be conducted with different types of orthodontic movements (32) and also with other materials, such as corticosteroids, which may interfere with the mechanism of root resorption (34).

Conclusion

Under the tested conditions, it may be concluded that the $Ca(OH)_2$-based root canal dressing and root filling materials used in this study had a favourable action on periapical tissue healing and repair of orthodontic root resorption in endodontically treated teeth in dogs.

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