A Comparison Study of Periapical Repair in Dogs' Teeth Using RoekoSeal and AH Plus Root Canal Sealers: A Histopathological Evaluation

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Abstract

The objective of the study was to evaluate the biocompatibility of RoekoSeal sealer Roeko (Dental Products, Langenau, Germany) with the periapical tissues of dogs and compare it with AH Plus sealer (Dentsply/De Trey, Konstanz, Germany). The pulps of 32 root canals were removed, the apical cementum layer perforated, the biomechanical preparation performed, and the root canals filled by lateral condensation technique. Ninety days after the surgery, the animals were euthanized, the bone with teeth removed, and the samples prepared for histopathological analysis. In group 1 (RoekoSeal Automix), deposition of mineralized tissue was observed, with complete newly mineralized apical formed tissue in 43.8% and partial sealing in 56.2%. In group 2 (AH Plus), in 12.5% there was complete newly mineralized apical formed tissue, in 75% the sealing was partial, and in 12.5% there was no sealing ($p < 0.05$). There were no differences between the groups in relation to the inflammatory infiltrate; thickness of the periodontal ligament; and the resorption of dentin, cementum or bone ($p > 0.05$). *(J Endod 2008;34:822–825)*

Key Words

AH Plus, biocompatibility, endodontic sealer, RoekoSeal

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The objective of endodontic therapy in cases of teeth with pulp necrosis and evident radiographically chronic periapical lesions is to eliminate microorganisms, their products, and subproducts and to achieve these results through a tridimensional hermetic seal of the root canal system (1–3). On the other hand, an important requirement for root canal sealers is their biological compatibility because they remain in close contact with living periapical tissues over a long period of time (4–7).

The most widely used root canal–filling materials are gutta-percha and zinc oxide eugenol. However, a new root canal sealer, silicon based-polydimethylsiloxane (RSA RoekoSeal Automix), has been developed by Roeko Dental Products (Langenau, Germany), and, according to the manufacturer, this material has a high sealing ability.

There have seen some studies of the physical and chemical properties of this product (1, 2). Wu et al. (8) measured in vitro the long-term leakage along the 4 mm remaining of the apical root canal filling using RSA RoekoSeal as a sealer. The results of this study showed that this sealer is dimensionally stable and prevents leakage for at least 1 year.

On the other hand, not only the physical and chemical properties needed to obtain a hermetic seal but also the biocompatibility of the filling material are important to success because the sealant may be responsible for completing histologic repair of periapical tissues after the root canal treatment. Therefore, complete periapical healing after root canal therapy may be influenced by the apical limit of the root canal obturation and the composition of the filling material (9).

There have not yet been any studies conducted that analyze the biological properties of RSA-RoekoSeal Automix Root Canal Sealer. Thus, the purpose of this study was to evaluate the biocompatibility with the periapical tissues of dogs' teeth when using this product as a root canal filling and to compare these results to the well-established biological properties of an epoxy-amine root canal sealer commercially called AH Plus (Dentsply/De Trey, Konstanz, Germany) (10–12).

Materials and Methods

All animal procedures were performed according to the protocols reviewed and approved by the Animal Care Committee of the University of São Paulo (Protocol #05.1.1010.53.7) in compliance with the applicable ethical guidelines and regulations of the international guiding principles for biomedical research involving animals. This methodology was based on the protocol recommended by the International Organization for Standardization standard Biological Evaluation of Dental materials (ISO 7405: 1997) (13).

This study was conducted on 32 root canals from the upper and lower premolars of two dogs (ages 12–18 months and weight 8–15 kg). The animals were anesthetized intravenously with sodium thiopental (30 mg/kg body weight, Thionembutal; Abbot Laboratories, São Paulo, Brazil). Standardized periapical radiographs were taken using a positioning device developed in our department for later comparison with standardized radiographs obtained after root canal treatment. After isolation of the dental arch with a rubber dam and antisepsis of the operative field with 2% chlorhexidine gluconate, coronal access to the pulp chamber was made.

TABLE 1. Groups, Composition of the Investigated Materials According to the Manufacturers, and Number of Roots Per Group

Groups	Investigated Materials	Component		Number of Roots
		A	B	
Group 1	RoekoSeal Automix	Polydimethylsiloxane Paraffin oil Silicon oil	Zirconium dioxide Hexachloroplatinum acid	16
Group 2	AH Plus	Diepoxide Calcium tungstate Zirconium oxide Silica Iron oxide pigment	1-Adamantane amine N,N-Dibenzyl-5-oxa-nonandiamine-1,9 TCD-diamine Calcium tungstate Zirconium oxide Silica Silicone oil	16

The apical patency of the root canals was established passively using a #15 K-file, and the work length was determined to approximately 2 mm short of the radiographic apex corresponding to the apical plateau level observed in dogs' teeth.

Next, the root pulps were removed using a #25 Hedströen file (Dentsply/Maillefer Instruments, Ballaigues, Switzerland), and afterward the root canals were irrigated with 1.0% sodium hypochlorite solution. The apical cementum layer observed in dogs' teeth was perforated with the sequential use of a size #15 K-file at the radiographic apex and then a # 25 K-file in order to create a standardized apical opening (14–16). After that, the biomechanical preparation was performed to the working length up to a #60 K-file (16) with irrigation with a 1.0% sodium hypochlorite solution.

After irrigation, the root canals were dried by suction and sterile paper points and then filled with 14.3% buffered EDTA (pH 7.4) for 3 minutes, irrigated with saline, and dried. After that, the root canals were filled by the lateral condensation technique using both root canal sealers and gutta-percha points. Because two variables should be tested in the same animal and in different quadrants, each hemiarch was submitted in an alternate manner to the experimental protocols (Table 1).

Both sealers were prepared according to the recommendations of the manufacturers and were introduced into the root canals with a size 60 gutta-percha cone as a master cone (Dentsply Ind Com Ltda, Petrópolis, RJ, Brasil). The lateral condensation technique was initiated with a digital spreader "C" (Dentsply/Maillefer Instruments), penetrating up to the apical third of the root canal and creating the space necessary to introduce auxiliary gutta-percha points (Dentsply Ind Com Ltda). Excess gutta-percha and sealer were removed with a heated instrument, and vertical compaction was applied. After the root canal filling, the occlusal cavities were restored with amalgam, which was condensed on a glass ionomer cement base (Vitremer; 3M Dental Products, St Paul, MN).

After a 90-day experimental period (±5 days) (13), the animals were euthanized with an intravenous overdose of sodium pentobarbital. The maxillas and the mandibles with teeth were dissected and sectioned in order to obtain individual roots, which were fixed in 10% buffered formalin for 72 hours.

After demineralization with EDTA in a microwave oven, the samples were prepared for histopathological analysis by staining with hematoxylin and eosin, Mallory trichrome, and Brown and Brenn stains. The samples were then examined under light microscopy by a calibrated examiner who was blinded to the treatment groups.

The sections were observed for determination of scores, and the following parameters were evaluated: (1) newly mineralized apical formed tissue: complete, partial, and absent; (2) periapical inflammatory infiltrate: absent, slight, moderate, and severe; (3) the apical periodontal ligament thickness: normal, slightly increased, moderately increased, and severely increased; (4) cementum resorption: absent and present; (5) dentin resorption: absent and present; and (6) bone tissue resorption: absent and present.

Statistical analysis of these histopathologic parameters was performed with the nonparametric Mann-Whitney *U* test at a 5% significance level. The parameters were evaluated subjectively by a calibrated examiner.

Results

Group 1: RoekoSeal Automix

Sixteen roots were evaluated (Fig. 1*A–C*). Deposition of mineralized tissue was observed, with complete newly mineralized apical formed tissue in 7 roots (43.8%) and partial sealing in 9 roots (56.2%). In the roots with partial mineralization, the connective tissue was normal with scarce inflammatory cells and blood vessels. The periodontal ligament was normal in 4 roots, slightly altered in 10 roots, and moderately altered in 2 roots. There was no cementary or osseous resorption. In the connective tissue, there were fibers, fibroblasts cells and collagen matrix.

Group 2: AH Plus

In two roots (12.5%), there was complete newly mineralized apical formed tissue, in 12 roots (75%) the mineralization was partial, and in 2 roots (12.5%) there was no mineralization (Fig. 1*D* and *E*). When the mineralization was partial, the connective tissue was extended to the interior of the root canal, and it contained fibroblasts and a high number of collagen fibers. The periapical ligament was normal in 2 specimens, slightly enlarged in 10 specimens, and moderately enlarged in 4 roots. The mineralized tissue, bone, and cementum were normal, containing cementoblasts and osteoblasts. Apical resorption was not observed.

The frequency of the histopathologic parameters evaluated for each group is reported in Table 2. The statistical analysis showed that in relation to the newly mineralized apical formed tissue, there were differences between the groups ($p < 0.05$), with better results in the RoekoSeal group. There were no differences between the groups in relation to the inflammatory infiltrate; the thickness of the periodontal ligament; or the resorption of dentin, cementum, or bone ($p > 0.05$).

Discussion

The biocompatibility of root canal sealers in relation to periapical tissues is important to achieve success in root canal therapy. The direct contact of these materials with this tissue can delay wound healing. A biocompatible root canal sealer should not delay the tissue repair process but instead support the repair and regenerative processes of the injured periradicular tissues (10, 17).

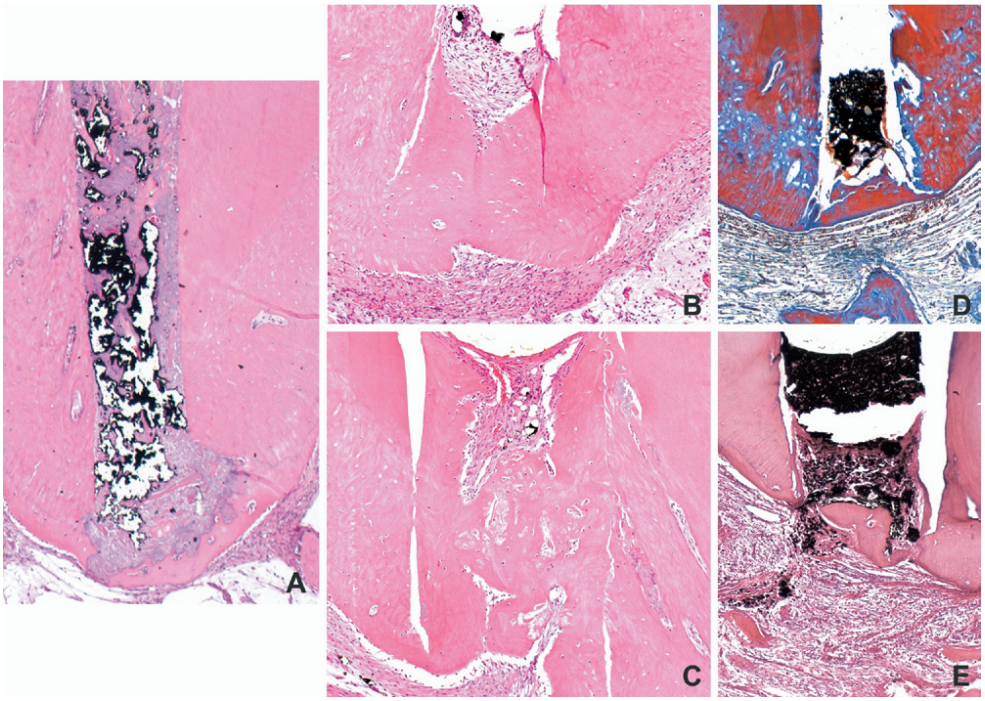

Figure 1. RoekoSeal: (*A*) complete biological sealing of the root apex with deposition of mineralized tissue (hematoxylin and eosin, 40×). (*B*) Partial biological sealing of the root apex. Normal connective tissue with intense fibrogenesis (hematoxylin and eosin, 64×). (*C*) Partial biological sealing of the root apex (hematoxylin and eosin, 64×). AH Plus: (*D*) complete biological sealing near the root canal sealer. Periodontal ligament with sparse inflammatory cells (Mallory trichrome, 64×). (*E*) Mineralized tissue formation beginning beneath the root canal sealer. Periodontal ligament moderately increased (hematoxylin and eosin, 64×).

Besides the fact that AHPlus and RSA RoekoSeal have presented similar results in this study, Miletic et al. (2005) (11) showed that AH Plus showed a strong cytotoxic effect immediately after mixing and after 24- and 48-hour setting periods in contact with human cervical carcinoma cells (HeLa) and mouse L929 fibroblasts. After 7 and 30 days of incubation, destruction was not observed within cell populations after the direct application of AH Plus. On the other hand, RSA did not show a toxic effect over any tested period.

TABLE 2. Frequency of the Histopathologic Findings in Each Group

Findings	Group 1: Roeko Seal	Group 2: AHPlus	p Value
Newly mineralized apical formed tissue			
Complete	7	2	<0.05*
Partial	9	12	
Absent	0	2	
Inflammatory infiltrate			
Absent	0	0	>0.05
Slight	16	16	
Moderate	0	0	
Severe	0	0	
Periodontal ligament space			
Normal	4	2	>0.05
Slightly increased	10	10	
Moderately increased	2	4	
Severely increased	0	0	
Dentin resorption			
Absent	16	16	>0.05
Present	0	0	
Cementum resorption			
Absent	16	16	>0.05
Present	0	0	
Bone resorption			
Absent	16	16	>0.05
Present	0	0	

*Statistically significant.

Öztan et al. (18) showed that the epoxy resin-based sealer AH Plus and the silicon-based sealer RSA have similar levels of cytotoxicity for mouse fibroblasts. Similar results were obtained by Schwarze et al. (19), who used immortalized 3T3 fibroblasts and primary human periodontal ligament fibroblasts. The discrepancy between these results and those of Miletic et al. (11) could be explained by the variation in experimental conditions.

Also, Al-Awadhi et al. (10) showed significantly less apoptotic activity in embryonic rat calvarial osteoblasts exposed to RoekoSeal than in cells exposed to Kerr's Pulp Canal Sealer (Sybron Kerr, Romulus, MI), suggesting a low cytotoxicity of that material. These results are in agreement with Bouillaguet et al. (20), who observed that the silicon-based material was less cytotoxic in direct contact with Balb/c 3T3 fibroblasts when compared with Kerr's Pulp Canal Sealer, TopSeal (Dentsply), and EndoREZ (Ultradent).

To our knowledge, this is the first study evaluating the periapical response of dogs' teeth using the RSA RoekoSeal as a root canal sealer. Besides the fact shown by Huumonen et al. (21) that the treatment outcome of root filling with RoekoSeal Automix 1 year after the treatment of apical periodontitis was satisfactory as evaluated by radiographs, we believe that the real response of the tissue needs to be evaluated microscopically as in this study. A canine model was chosen because it is the most widely used experimental model in biological research, and it has been shown that the pulpal, apical, and periapical healing process in dogs is similar to that in humans (22).

AH Plus sealer is commonly used in clinical practice. The animal experimental studies that have examined the effect of periradicular vital tissue exposure reported tissue repair activity (23), as shown in this study.

According to this research, the newly introduced sealer RSA RoekoSeal displayed satisfactory biological response when compared with the effect of AH Plus under the present experimental conditions.

References

1. Wu MK, Tigos E, Wesselink PR. An 18-month longitudinal study on a new silicon-based sealer, RSA RoekoSeal: a leakage study in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:499–502.
2. Schafer E, Zandbiglari T. Solubility of root-canal sealers in water and artificial saliva. Int Endod J 2003;36:660–9.
3. Kazemi RB, Safavi KE, Spangberg LS. Dimensional changes of endodontic sealers. Oral Surg Oral Med Oral Pathol 1993;76:766–71.
4. Leonardo MR, Silva LA, Utrilla LS, Leonardo RT, Consolaro A. Effect of intracanal dressings on repair and apical bridging of teeth with incomplete root formation. Endod Dent Traumatol 1993;9:25–30.
5. Leonardo MR, Almeida WA, Ito IY, da Silva LA. Radiographic and microbiologic evaluation of post treatment apical and periapical repair of root canals of dogs' teeth with experimentally induced chronic lesion. Oral Surg Oral Med Oral Pathol 1994;78:232–8.
6. Leonardo MR, Almeida WA, Silva LA, Utrilla LS. Histopathological observations of periapical repair in teeth with radiolucent areas submitted to two different methods of root canal treatment. J Endod 1995;21:137–41.
7. Trope M, Delano EO, Orstavik D. Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. J Endod 1999;25:345–50.
8. Wu MK, van der Sluis LW, Wesselink PR. A 1-year follow-up study on leakage of single-cone fillings with RoekoRSA sealer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:662–7.
9. Leonardo MR, Silva LAB, Assed S, Ether SS. Calcium hydroxide root canal sealers— histopatologic evaluation of apical and periapical repair after endodontic treatment. J Endod 1997;27:428–32.
10. Al-Awadhi S, Spears R, Gutmann JL, Opperman LA. Cultured primary osteoblast viability and apoptosis in the presence of root canal sealers. J Endod 2004;30:527–33.
11. Miletic I, Devci N, Anic I, Borcic J, Karlovic Z, Osmak M. The cytotoxicity of RoekoSeal and AH Plus compared during different setting periods. J Endod 2005;31:307–9.
12. Sousa CJ, Sousa CJ, Montes CR, Pascon EA, Loyola AM, Versiani MA. Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ, and Epiphany root canal sealers. J Endod. 2006;32:656–62.
13. ISO 7405: Dentistry–preclinical evaluation of biocompatibility of medical devices used in dentistry–test methods for dental materials. Switzerland: International Organization for Standardization; 1997.
14. Holland R, Otoboni Filho JA, de Souza V, Nery MJ, Bernabe PF, Dezan E Jr. A comparison of one versus two appointment endodontic therapy in dogs' teeth with apical periodontitis. J Endod 2003;29:121–4.
15. Holland R, Mazuqueli L, de Souza V, Murata SS, Dezan Júnior E, Suzuki P. Influence of the type of vehicle and limit of obturation on apical and periapical tissue response in dogs' teeth after root canal filling with mineral trioxide aggregate. J Endod 2007;33:693–7.
16. Leonardo MR, Barnett F, Debelian GJ, Pontes Lima RF, Silva LAB. Root canal adhesive filling in dog's teeth with or without coronal restoration: a histopathological evaluation. J Endod 2007;33:1299–1303.
17. Leonardo MR. Endodoncia: tratamiento de conductos radiculares. Principios técnicos y biológicos. São Paulo: Artes Médicas-Latino América; 2005:503–6.
18. Öztan MD, Yilmaz S, Kalayci A, Zaimoglu L. A comparison of the *in vitro* cytotoxicity of two root canal sealers. J Oral Rehabil 2003;30:426–9.
19. Schwarze T, Leyhausen G, Geurtsen W. Long-term cytocompatibility of various endodontic sealers using a new root canal model. J Endod 2002;28:749–53.
20. Bouillaguet S, Wataha JC, Lockwood PE, Galgano C, Golay A, Krejci I. Cytotoxicity and sealing properties of four classes of endodontic sealers evaluated by succinic dehydrogenase activity and confocal laser scanning microscopy. Eur J Oral Sci 2004;112:182–7.
21. Huumonen S, Lenander-Lumikari M, Sigurdsson A, Orstavik D. Healing of apical periodontitis after endodontic treatment: a comparison between a silicone-based and a zinc oxide–eugenol-based sealer. Int Endod J 2003;36:296–301.
22. Citrome GP, Kaminsky EJ, Heuer MA. A comparative study of tooth apexification in the dog. J Endod 1979;5:207–9.
23. Leonardo MR, Silva LA, Almeida WA, Utrilla LS. Tissue response to an epoxy resin-based root canal sealer. Endod Dent Traumatol 1999;15:28–32.