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Analysis of the healing process in delayed tooth replantation after root canal filling with calcium hydroxide, Sealapex and Endofill: a microscopic study in rats

Márcia Regina Negri, Sônia Regina Panzarini, Wilson Roberto Poi, Celso Koogi Sonoda, Jéssica Lemos Gulinelli, Célia Tomiko Matida Hamata Saito

Department of Surgery and Integrated Clinics, School of Dentistry of Araçatuba, São Paulo State University (UNESP), Araçatuba, SP, Brazil

Correspondence to: Prof. Dr Sônia Regina Panzarini, Departamento de Cirurgia e Clínica Integrada, Disciplina de Clínica Integrada, Faculdade de Odontologia do Campus de Araçatuba, UNESP, Rua José Bonifácio 1193, CEP: 1601-050, Araçatuba, SP, Brazil
Tel.: +55 18 3620 3240 or +55 18 3622 5693
Fax: +55 3636 3332
e-mail: panzarin@foa.unesp.br

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Abstract – The major concern in the therapeutics of tooth replantation refers to the occurrence of root resorption and different approaches have been proposed to prevent or treat these complications. The purpose of this study was to evaluate tissue response to delayed replantation of anterior rat teeth treated endodontically using calcium hydroxide, Sealapex, and Endofill without the placement of gutta-percha cones. Thirty rats had their right upper incisor extracted and maintained in dry storage for 60 min. After removal of the dental papilla, enamel organ, pulp tissue, and periodontal ligament remnants, the teeth were immersed in 2% sodium fluoride phosphate acidulated, pH 5.5, for 10 min. The root canals were dried with absorbent paper points and the teeth were assigned to three groups (n = 10) according to the filling material. Group I – calcium hydroxide and propyleneglycol paste, Group II – Sealapex, and Group III – Endofill. The sockets were irrigated with saline and the teeth were replanted. Replacement resorption, inflammatory resorption and ankylosis were observed in all groups. Although the occurrence of inflammatory resorption was less frequent in Group I, there were no statistically significant differences among the groups. It may be concluded that compared to the paste, filling the root canals with Sealapex and Endofill sealers without the placement of gutta-percha cones did not provide better results.

The major concern in the management of teeth replanted after traumatic avulsions is the occurrence of root resorption (1, 2). Different approaches have been proposed to prevent, delay or resolve this posttreatment complication. According to Andreasen (1, 2), inflammatory and replacement root resorption are directly related to the periodontal damage caused by long extra-alveolar periods and presence of contamination. Pulp necrosis is expected in cases of delayed replantation, especially in mature teeth. Therefore, the accomplishment of high-quality endodontics is of paramount importance and plays a key role on the success of replantation (2–5).

Traditionally, it has been recommended that replanted mature teeth are treated endodontically 7–14 days after replantation, with placement of interappointment intracanal dressings, as appropriate (1–6). Calcium hydroxide fulfills all requirements of an intracanal dressing because, in addition to its antimicrobial activity, this material neutralizes bacterial toxins and induces tissue repair (7). Nevertheless, calcium hydroxide takes 2–3 weeks to produce dentin alkalinization and requires periodical changes to maintain its beneficial properties (7, 8). Owing to the biologic properties of this material, including its capacity to induce tissue calcification and excellent bactericidal action, calcium hydroxide-containing root canal sealers have been launched to the market. Sealapex is an endodontic sealer that contains calcium oxide, which, in contact with water, forms calcium hydroxide (9). Several studies have demonstrated the biocompatibility of this root filling material, which induces only a mild inflammatory reaction when in contact with the periapical tissues (9–13). The solubility of Sealapex is considered a negative characteristic of this material. However, it may be advantageous in cases of delayed tooth replantation in which exposure of the root filling material is unavoidable because of the occurrence of replacement resorption. It has been suggested that the solubilization of Sealapex may be related to its slow and continuous release of calcium and hydroxyl ions (11, 14, 15). This constant ion leaching produces alkalinization of the medium, which is necessary to stimulate tissue mineralization and maintain the antimicrobial potential (11, 13).

Zinc oxide and eugenol are basic components of most commercially available root filling materials, such as Endofill, which is one of the most traditional root canal sealers in the market (16). Because it is a popular material with well-defined physicochemical properties, Endofill has been extensively investigated, including this study in which it was used as a comparison standard (16–21). In addition, this material has been frequently used for root canal filling in cases of tooth replantation (3, 22).

When replacement root resorption occurs in endodontically treated replanted teeth, there is exposure of gutta-percha cones and endodontic sealer. Radiographically, gutta-percha cones are identified within the bone tissue after complete root resorption. Based on these findings, the possibility of using an endodontic sealer (with no gutta-percha filling) that has antimicrobial properties and may be resorbed during the process of replacing dental tissue by bone tissue has been speculated. The purpose of this study was to evaluate tissue response to delayed replantation of rat teeth treated endodontically using calcium hydroxide, Sealapex, and Endofill without the placement of gutta-percha cones.

Materials and methods

The research proposal was reviewed by the Ethics in Animal Research Committee of the School of Dentistry of Araçatuba, São Paulo State University, Brazil, and the study design was approved.

Thirty male Wistar rats (*Rattus norvegicus, albinus*) weighing 250–300 g were used in this study. The animals were fed ground solid ration (Ração Ativada Produtor, Anderson & Clayton S.A., São Paulo, SP, Brazil) and water *ad libitum*, except for the preoperative 12 h. The animals received an intramuscular injection of xilazine chlorhydrate (Anasedan; AgriBrands do Brasil Ltda., Jacareí, SP, Brazil; 0.03 ml/100 g body weight) to attain muscular relaxation and were then anesthetized with ketamine chlorhydrate (Dopalen; AgriBrands do Brasil Ltda.) at a dose of 0.07 ml/100 g body weight. After anesthesia, asepsis of the anterior maxilla was performed with iodine polyvinylpyrrolidone (Riodeine; Ind. Farmacêutica Rioquímica Ltda., Rio de Janeiro, RJ, Brazil), followed by syndesmotomy, luxation and non-traumatic extraction of the maxillary right incisor of all animals.

The extracted teeth were held by their crowns, fixed on a red wax plate and kept dry at room temperature for 60 min. Next, the dental papilla and enamel organ of each tooth were removed with a #15 scalpel blade (Embramac Exp. e Imp., Campinas, SP, Brazil). The pulp tissue was extirpated through a retrograde route with a slightly curved #35 K-file (25 mm; Sybron Kerr Corporation, Orange, CA, USA). Root canals were irrigated with saline (Ariston Ind. Quím. e Farm. Ltda, São Paulo, SP, Brazil) and fluid was aspirated with a 25 × 6 needle attached to a disposable syringe.

The teeth had their root surface gently scraped with a #15 scalpel blade (Embramac Exp. e Imp.) mounted on a scalpel handle to remove periodontal ligament (PDL) remnants mechanically. Each root was scraped only once with the blade positioned perpendicularly to the long axis of the lingual surface and moved in a crown-apex direction. After PDL removal, the teeth were immersed in 20 ml of 2% acidulated-phosphate sodium fluoride, pH 5.5 (Apothicário Farmácia de Manipulação, Araçatuba, SP, Brazil) for 10 min. Thereafter, fluid aspiration was performed with a 25×6 needle coupled to a disposable syringe and the root canals were dried with absorbing paper points (Dentsply Industria e Comércio Ltda, Petrópolis, RJ, Brazil) and were assigned to three groups ($n = 10$), according to the filling material. Group I – The root canals were filled with a calcium hydroxide-based paste (5 ml propyleneglycol, 5 g calcium hydroxide, 2 g zinc oxide, and 0.015 g colophony; Discipline of Endodontics, School of Dentistry of Araçatuba), which was packed in anesthetic cartridges and injected via retrograde route with a long curved blunt-tipped needle (G27; Terumo Corporation, Tokyo, Japan) attached to a Carpule syringe. Group II – The canals were filled with Sealapex (Sybron-Kerr, Romulus, MI, USA) via retrograde route using an insulin syringe. The sealer was mixed and handled according to the manufacturer's instructions. Group III – The canals were filled with Endofill (Dentsply Industria e Comércio Ltda) in the same way as described for Group II. The sealer was mixed and handled according to the manufacturer's instructions.

The sockets were gently irrigated with saline and the teeth were replanted. All animals received a single intramuscular dose of benzathine G penicillin 20 000 IU (Fort Dodge® Animal Health Ltda., Campinas, SP, Brazil).

Sixty days after replantation, the rats were sacrificed by anesthetic overdose. The obtained specimens were fixed in 10% formalin for 24 h and decalcified in a 4.13% EDTA solution, pH 7.0. After decalcification, the specimens were embedded in paraffin and longitudinal 6-μm-thick sections were obtained and stained with hematoxylin and eosin for histologic and histomorphometric analyses under optical microscopy.

Only the lingual surface of the roots was examined because in rats the PDL fibers attach only to this region of the root. The sections were analyzed with respect to gingival mucosa, PDL, alveolar bone, cementum and dentin characteristics, as well as to the occurrence of inflammatory resorption, replacement resorption, and ankylosis.

For histomorphometric analysis and measurement of the root area affected by the resorption process, the images of the longitudinal root sections were divided into three thirds (cervical, middle, and apical) using a compass, a ruler and a fine pen. The middle third was selected for the measurements because this region is not damaged by the surgical procedures. The cervical and apical thirds, on the other hand, are affected by the grasping action of the forceps and cutting action of the scalpel blade during tooth extraction and dental papilla removal, respectively. Images were captured using a digital video camera (JVC TK-1270 Color Video Camera, Tokyo, Japan) coupled to a Carl Zeiss microscope (Axiolab) and connected to a microcomputer and using Microsoft VidCap video capture software (Microsoft Corp., Redmond, WA, USA). Two image captures (720 × 480 pixel) were made to cover the entire middle third. These images were first recorded as figures (Tif 24) and further joined at 200× zoom using an image-editing software (Corel Photo Paint 10; Corel Corporation, Ottawa, ON, Canada). For histomorphometric analysis of the middle root third, the final images were analyzed using ImageLab® 2001 image-analysis software (Diracom Bio Informática, Vargem Grande do Sul, SP, Brazil) for determination of the resorption areas and ankylosis perimeter. The obtained data were entered in Excel software (Microsoft Corp., Redmond, WA, USA) for statistical analysis, taking into account the total root area and the areas of inflammatory and replacement root resorption in the middle third. Ankylosis was determined by measuring the total perimeter of the lingual root surface and the perimeter of the ankylosed areas. Ankylosed areas were defined as those in which the bone tissue was juxtaposed with the cementum. Identification and selection of the resorption areas were performed by comparing the digital images to the images of the histological sections obtained under optical microscopy.

For statistical analysis, scores from 1 to 4 were attributed to inflammatory resorption, replacement resorption, and ankylosis, 1 being the best result, 4 being the worst result and 2 and 3 occupying intermediate positions. *Root resorption area*: the areas of inflammatory and replacement root resorption were measured in representative slides. A 4-point scoring system was used, as follows: 1 – No resorption; 2 – 0.1% to 50% of the area with resorption; 3 – 51%–99% of the area with resorption; 4 – 100% of the area with resorption. *Ankylosis perimeter*: the perimeter of the ankylosed areas was measured in representative slides. A 4-point scoring system was used, as follows: 1 – Absence of ankylosis; 2 – Few areas of ankylosis; 3 – 50% of the analysis field with ankylosis; 4 – 100% of the analysis field with ankylosis. Means and standard deviations of the scores attributed to each type of resorption with each type of root canal filling were calculated. Data obtained for the different groups were analyzed statistically by Kruskal–Wallis non-parametric test at 5% significance level.

Results

The animals endured adequately all experimental procedures. However, during the course of the study, two animals per group were lost, which reduced the sample size to 24 ($n = 8$).

The results were drawn from a qualitative analysis after observation of the following structures: gingival mucosa, PDL space, cementum, dentin, alveolar wall and bottom of the socket, 60 days after replantation.

Group I (calcium hydroxide; *n* = 8)

The gingival mucosa epithelium was close to the cemental surface, below the cementoenamel junction. The subjacent connective tissue exhibited fibroblasts and few lymphocytes. The PDL space was filled by a fibrous connective tissue, with fibers disposed parallel to the root surface and presence of newly formed bone tissue. Cementum and dentin were intact only in few areas of root surface. In most specimens, active replacement resorption was observed along the three root thirds and few areas resorbed by an inflammatory process with presence of numerous lymphocytes (Fig. 1). Areas where the bone tissue was in direct contact with the cementum were also observed. The alveolar wall presented bone apposition, causing the narrowing and/or compete filling of the PDL space (Fig. 2). Newly formed bone trabeculae were observed at the bottom of the socket.

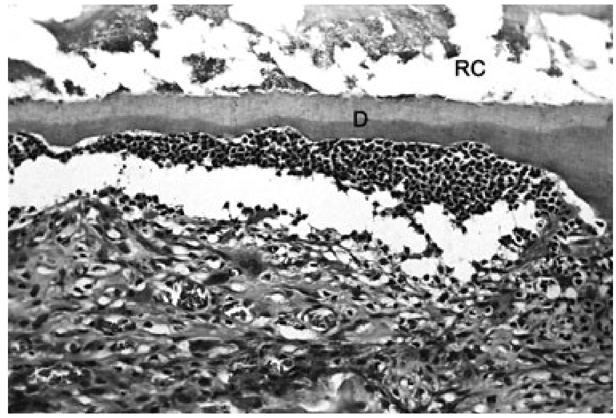

Fig. 1. Group I (calcium hydroxide) – area of inflammatory root resorption with presence of numerous lymphocytes. RC, root canal; D, dentin. H&E, original 160×.

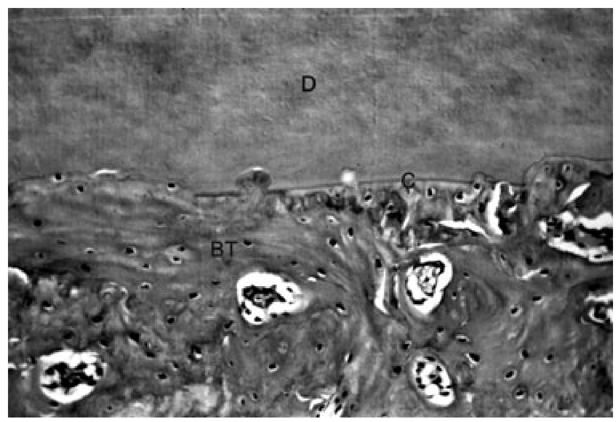

Fig. 2. Group I (calcium hydroxide) – PDL replaced by newly formed bone tissue (BT) showing areas of direct contact with the cementum (C). D, dentin. H&E, original 160×.

Group II (Sealapex; *n* = 8)

The gingival mucosa epithelium was located below the cementoenamel junction with subjacent tissue exhibiting fibroblasts and lymphocytes. In few areas, the PDL space was filled by a fibrous connective tissue, with fibers arranged parallel to the root surface. In three specimens, macrophages with cytoplasmatic inclusions containing particles of the filling material were observed. In only one specimen, cementum and dentin appeared preserved in most part of the root. In the other specimens of this group, ongoing processes of replacement of root tissue by bone tissue (Fig. 3) and areas of inflammatory root resorption with presence of numerous lymphocytes (Fig. 4) were observed. Juxtaposition of cementum with bone tissue was noted in few areas of root surface. Along the three alveolar root thirds, there was bone apposition on the alveolar wall causing narrowing of the PDL space.

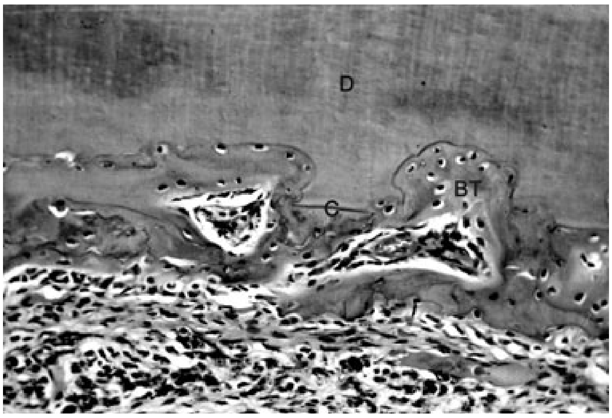

Fig. 3. Group II (Sealapex) – area of replacement resorption (arrow) and ankylosis. D, dentin; C, cementum; BT, bone tissue. H&E, original 160×.

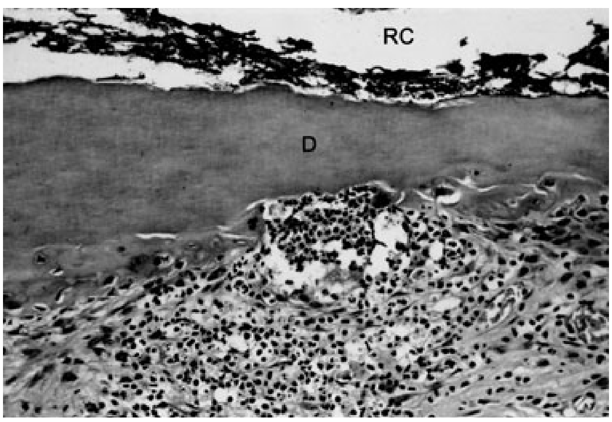

Fig. 4. Group II (Sealapex) – area of inflammatory root resorption with presence of numerous lymphocytes. RC, root canal; D, dentin. H&E, original 160×.

The presence of newly formed bone trabeculae were observed at the bottom of the socket. Connective tissue with a discrete number of fibroblasts was noted in contact with the filling material. Numerous macrophages exhibiting particles of Sealapex sealer in their cytoplasm were also found.

Group III (Endofill; *n* = 8)

The gingival mucosa epithelium was located below the cementoenamel junction and the subjacent connective tissue presented a small number of fibroblasts and lymphocytes. In few areas, the PDL space was filled by a fibrous connective tissue, with fibers disposed parallel to the root surface. In five specimens, this connective tissue exhibited several lymphocytes close to the areas of dentinal and cemental resorption (Fig. 5). In eight specimens, cementum and dentin presented areas of resorption. Direct contact of the bone tissue with the cementum was observed in few areas of root surface (Fig. 6). In all specimens, the alveolar wall exhibited bone apposition, causing the narrowing and/or compete filling of the PDL space. The presence of newly formed

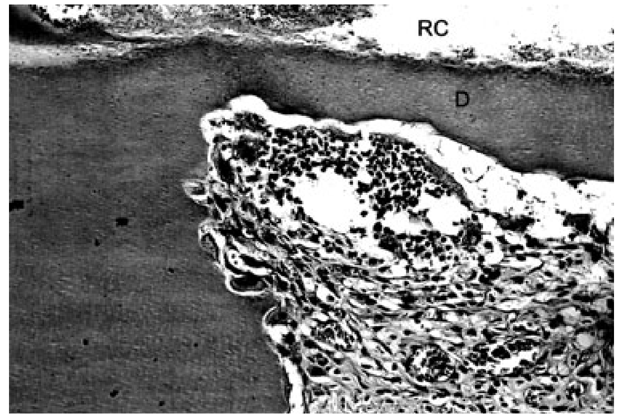

Fig. 5. Group III (Endofill) – areas of inflammatory root resorption. RC, root canal; D, dentin. H&E, original 160×.

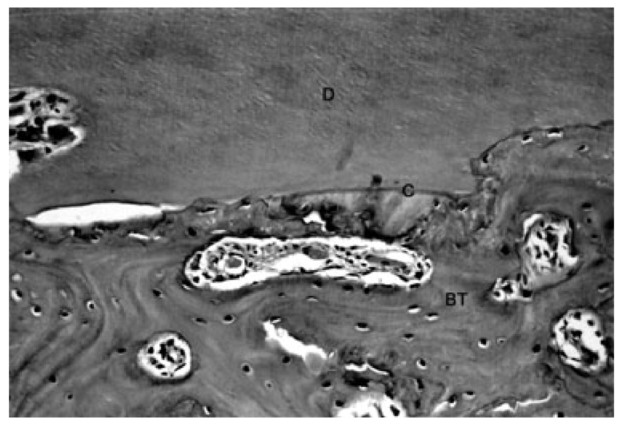

Fig. 6. Group III (Endofill) – PDL space filled by newly formed bone tissue (BT) with few areas of direct contact with the cementum (C). D, dentin. H&E, original 160×.

bone trabeculae in contact with the filling material was noted at the bottom of the socket as well as connective tissue with a large number of lymphocytes.

The statistical analysis of the data obtained from the histomorphometric evaluation showed no significant difference among the groups (root filling materials) (Kruskal–Wallis test: KW = 3.067, P = 0.2158).

Discussion

The replantation of exarticulated teeth should be performed within the shortest possible interval because the extra-alveolar period is inversely proportional to the likelihood of maintenance of the replanted tooth without sequelae (23, 24). In addition to extra-oral time, other factors that influence the healing process include the storage medium in which the avulsed tooth is kept until the moment of replantation (25), use of retention (2), root surface treatment (2, 23, 26), root canal therapy (1–6, 21) and antibiotic therapy (2, 6).

In the present study, dry storage medium and 1-h extra-alveolar period were used to simulate the unfavorable clinical conditions under which most teeth are replanted (26). No retention was placed after replantation because, according to Okamoto et al. (27), the use of splints not only interferes negatively with the healing process in the experimental animal model used in this study. Root surface treatment consisted of mechanical PDL removal followed by immersion in 2% acidulated-phosphate sodium fluoride (6). These phases of the treatment were standardized for all groups, as the goal of this study was to investigate the interference of the root canal filling material.

The literature has reported a high prevalence of delayed tooth replantation (26) and, when the PDL space cannot be preserved, a resorption process is likely to develop and lead to complete root resorption causing tooth loss (23). This type of resorption was observed in all groups of the present study due to the mechanical removal of PDL remnants.

The main etiologic agent in inflammatory root resorption is pulp necrosis associated with PDL damage and contamination (28). According to Holland et al. (29), the bacteria lodged in the dentinal tubules do not prevent PDL repair as long as the area is small sized and PDL is preserved. Therefore, case prognosis in tooth replantation is entailed to endodontic treatment (1–4, 28, 30) because the PDL is compromised and sometimes absent, and the bacteria present in the root canal system may interfere with the healing process. This was one of the reasons why this study investigated root canal sealers that could favor the repair, especially concerning the control of contamination.

The use of calcium hydroxide-based pastes as temporary root canal filling materials may prevent or arrest inflammatory root resorption, especially because of calcium hydroxide's capacity of neutralizing bacterial toxins and antimicrobial action (7, 31, 32). This fact was confirmed by the findings of the present study, as Group I presented fewer areas of inflammatory resorption than Groups II and III.

The velocity of the ionic dissociation of calcium hydroxide is related to the type of vehicle used. For this reason, the hydrosoluble vehicles should be the first choice in cases of traumatic injuries (7, 33) in order to alkalinize the medium before the beginning of the inflammatory root resorption process, which occurs within 2 weeks approximately (2, 4). It is likely that this fact also contributed for the better results observed in Group I, in which propyleneglycol was the vehicle used for preparation of the calcium hydroxide paste.

Sealapex, the other root canal sealer used in this study, contains 25% calcium oxide in its composition, which, when hydrated, is converted into calcium hydroxide (9, 11, 13).

In the present study, Group II (Sealapex) showed a larger number of inflammatory root resorption areas than the other groups. A possible explanation may be the fact that the release of hydroxyl ions from this material is slow (11, 14, 15) and hence the alkalinization occurs within a longer time, which retards the antimicrobial action in relation to onset of the inflammatory resorption.

Another issue that might explain the less favorable results of Sealapex compared to the calcium hydroxide paste would be related to sealer's composition, as calcium hydroxide is formed from a chemical reaction between the calcium oxide present in the sealer and water (9). This amount of calcium hydroxide is significantly lower than that of the paste, which contains only this substance. Regarding its biocompatibility and solubility, the analysis of the histologic sections in which the material was in direct contact with the periapical tissues revealed the presence of a fibrous connective tissue with macrophages phagocyting the material. However, few acute inflammatory cells were observed, which characterizes the low toxicity of this material (9, 10, 12, 14, 16, 17).

Other types of root canal sealers used in cases of tooth replantation are zinc-oxide-and-eugenol-based cements (22), such as Endofill, which was used in the present study as a comparison standard with Sealapex and the calcium hydroxide-based paste. Several divergences are observed when data from studies using zinc-oxide-and-eugenol-based sealers are compared, mainly because of differences in the powder-to-liquid ratio (17, 20, 34) and the time of postoperative evaluation (17, 35).

Without an accurate powder-liquid proportion, the amount of eugenol for portion of material is not precise, which may lead to the addition of excessive eugenol to the final mixture.

In this study, Group III (Endofill) showed less inflammatory root resorption than Group II (Sealapex). This may be explained by the fact that, unlike Sealapex (11, 13, 36, 37), Endofill contains eugenol, which provides an immediate antibacterial effect (18–20). In spite of their satisfactory physicochemical properties and antimicrobial action (18, 19), the zinc-oxide-and-eugenol-based sealers did not present a favorable biological behavior.

A large number of lymphocytes was observed in the histologic sections in which Endofill was in direct contact with the tissues, which confirms its toxicity (16, 17, 38).

The release of eugenol from the sealer occurs by hydrolysis of eugenolate. This indicates that tissue humidity determines how much eugenol will be leached (20). In cases of delayed replantation with external root resorption, the material will remain permanently exposed to the tissues.

Until a substitute to the PDL is not found, the occurrence of replacement root resorption in cases of delayed tooth replantation is expected and will cause exposure of the root canal filling material to the tissues. The use of root canal sealers without gutta-percha filling that, at the same time, are biocompatible, resorbable and present antimicrobial properties is an interesting approach to be further investigated in the search for a better repair. The results of this study cannot be fully extrapolated to the human situation and this research line should be further explored in future studies before this technique can be recommended for routine clinical use.

Conclusions

The findings of this study showed a good tissue response of the use of calcium hydroxide-based paste as a temporary root canal filling material in cases of delayed replantation. Compared to the paste, filling the root canals with Sealapex and Endofill sealers without the placement of gutta-percha cones did not provide better results.

References

1. Andreasen JO, Kristerson L. The effect of extra-alveolar root filling with calcium hydroxide on periodontal healing after replantation of permanent incisors in monkeys. J Endod 1981;7:349–54.
2. Andreasen JO, Andreasen FM, Andersson L. Textbook and color atlas of traumatic injuries to the teeth, 4th edn. Oxford: Blackwell Munksgaard; 2007.
3. Hammarström L, BlomLöf L, Feiglin B, Lindskog S. Effect of calcium hydroxide treatment on periodontal repair and root resorption. Endod Dent Traumatol 1986;2:184–9.
4. Trope M, Yesilsoy C, Koren L, Moshonov J, Friedman S. Effect of different endodontic treatment protocols on periodontal repair and root resorption of replanted dog teeth. J Endod 1992;18:492–6.
5. Trope M, Moshonov J, Nissan R, Buxt P, Yesilsoy C. Short vs. long-term calcium hydroxide treatment of established inflammatory root resorption in replanted dog teeth. Endod Dent Traumatol 1995;11:124–8.
6. Flores MT, Andreasen JO, Bakland LK, Feiglin B, Gutmann JL, Oikarinen K et al. Guidelines for the evaluation and management of traumatic dental injuries. Dent Traumatol 2001;17:193–6.
7. Estrela C, Holland R. Calcium hydroxide: study based on scientific evidences. J Appl Oral Sci 2003;11:269–82.
8. Nerwich A, Fidgor 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.
9. Holland R, Souza V. Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. J Endod 1985;11:535–43.
10. Silva LAB, Leonardo MR, Faccioli LH, Figueiredo F. Inflammatory response to calcium hydroxide based root canal sealers. J Endod 1997;23:86–90.
11. Tagger M, Tagger E, Kfir A. Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. J Endod 1988;14:588–91.
12. Canova GC, Taveira LAA, Dezan Jr E, Nishiyama CK. Endemogenic test study on the flogogen capacity of four root canal filling cements. Rev Fac Odontol Bauru 2002;10:128–33.
13. Duarte MAH, Demarchi AC, Giaxa MH, Kuga MC, Fraga SC, de Souza LC. Evaluation of pH and calcium ion release of three root canal sealers. J Endod 2000;26:389–90.
14. Trönstad L, Barnett F, Flax M. Solubility and biocompatibility of calcium hydroxide – containing root canal sealers. Endod Dent Traumatol 1988;4:152–9.
15. Schafer E, Zandbiglari T. Solubility of root canal sealers in water and artificial saliva. Int Endod 2003;36:660–9.
16. Leonardo RT, Consolaro A, Carlos IZ, Leonardo MR. Evaluation of cell culture cytotoxicity of five root canal sealers. J Endod 2000;26:328–30.
17. Holland R. A histological comparison of periapical inflammatory and neural responses to two endodontic sealers in the ferret. Arch Oral Biol 1994;4:539–44.
18. Hume RW. The pharmacologic and toxicological properties of zinc oxide-eugenol. JADA 1998;113:789–91.
19. Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and oxide-eugenol. Oral Surg Oral Med Oral Pathol 1992;73:729–37.
20. Hume WR. An analysis of the release and the diffusion through dentin of eugenol from zinc oxide-eugenol mixtures. J Dent Res 1984;63:881–4.
21. Dunsha T, Hovlland EJ. Evaluation of long-term calcium hydroxide treatment in avulsed teeth: an vivo study. Int Endod J 1995;28:7–11.
22. Pavek DI, Radtke PK. Posreplantation management of avulsed teeth: an endodontic literature review. Gen Dent 2000;48:176–81.
23. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. Endod Dent Traumatol 1995;11:76–89.
24. Hammarström L, Blomöf L, Lindskog S. Dynamics of dentoalveolar ankylosis and associated root resorption. Endod Dent Traumatol 1989;5:163–75.
25. Ashkenazi M, Sarnat H, Keila S. In vitro viability, mitogenicity and clonogenic capacity of periodontal ligament cells after storage in six different media. Endod Dent Traumatol 1999;15:149–56.
26. Panzarini SR, Saad-Neto M, Sonoda C, Poi WR, Perri de Carvalho AC. Dental avulsion in young and adult patients in the region of Araçatuba. Rev Assoc Paul Cir Dent 2003;57:27–31.
27. Okamoto T, Barbieri CM, Okamoto R, Sonoda CK. Replantation mediato de incisio superior de rato. Intereferência da imobilização sobre a reparação das estruturas dentais e peridentais. Rev Salusvita 1998;17:155–64.
28. Ehnevid H, Jansson L, Lindskog S, Weintraub A, BolmLöf L. Endodontic pathogens: propagation of infection through patent dentinal tubules in traumatized monkey teeth. Endod Dent Traumatol 1995;11:229–34.
29. Holland R, Otoboni Filho JA, Bernabé PFE, Nery MJ, Souza V, Berbert A. Effect of root canal status on periodontal healing after surgical injury in dogs. Endod Dent Traumatol 1994;10:77–82.
30. Andreasen JO, Borum MK, Jacobsen HL. Replantation of 400 avulsed permanent incisors. 2. Factors related to pulpal healing. Endod Dent Traumatol 1995;11:59–68.
31. Tronstad L, Andreasen JO, Hasselgreen G, Kristerson L. pH changes in dental tissues after root filling with calcium hydroxide. J Endod 1981;7:17–21.
32. Safavi K, Nichols FC. Effect of calcium hydroxide on bacterial lipopolysaccharide. J Endod 1993;19:76–8.
33. Holland R, González AC, Nery MJ, Souza V, Otoboni Filho JA, Bernabé PFE. Efecto de los medicamentos colocados em el interior del conducto, hidrosolubles y no hidrosolubles em el proceso de reparación de dientes de perro con lesión periapical. Endodontica 1999;17:90–100.
34. Holland R, Souza V, Abdalla T, Russo MC. Sealing properties of some root filing materials evaluated with radioisotope. Aust Dent J 1974;19:322–5.
35. Mittal M, Chandra S. Comparative tissue toxicity evaluation of four endodontic sealers. J Endod 1995;21:622–4.
36. Silva LAB. Calcium hidroxide root canal sealers: evaluation of pH, calcium ion concentration and condutivity. Int Endod J 1997;30:205–9.
37. Shalhav M, Fuss Z, Weiss E. Antimicrobial activity of calcium hydroxide – containing endodontic sealers on *Streptococcus faecalis* in vitro. Int Endod J 1996;29:208.
38. Trönstad L. Solubility and biocompatibility of calcium hydroxide-containing root canal sealers. Endod Dent Traumatol 1988;4:152–9.