

*Artigo Original de Pesquisa*

# Apical and periapical tissues responses after root canal obturation with two calcium hydroxid based sealers in dog's teeth<sup>1</sup>

## Reações teciduais apicais e periapicais quando da obturação de canais radiculares de cães por dois cimentos contendo hidróxido de cálcio

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### Abstract

**Introduction:** At the ending of endodontic treatment, is very important that the choice of root canal's sealer will be done by biological compatibility criterial, which could promote less inflammation, turning quicker and more effective the healing process. **Objective and methodology:** The aim of this study was to evaluate the apical and periapical tissues reactions in dog's teeth, after pulpectomy and root canal's cleaning and shaping, which limit was the fisiological barrier of delta apical, and filling with gutta-percha and two calcium hydroxide based endodontics sealers, Sealapex® and Apexit®, by those hystopatological events in observation periods of 7, 21 and 45 days, making a comparison of inflammatory reactions and reparacional evolution that both materials promoted. Past all periods, the dogs were killed and histological cuts of teeth were obtained. **Results:** Through

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qualitative-quantitative analysis, Sealapex showed inflammatory level among mild and moderate in all periods, having a great improvement in healing process at 45 days. There was a biggest dispersion of Apexit in periapical tissue, which caused an intense macrophage activity, raising inflammatory level in last period. **Conclusions:** Regardless of the sealer used, there were observed necrosis in a few of delta apical's ramifications, being mostly in tooth filling by Apexit. In both ways, Sealapex showed best results than Apexit, about increased inflammatory reaction and healing process.

#### **Palavras-chave:**

endodontia; cimentos obturadores; compatibilidade biológica.

## **Resumo**

**Introdução:** No término do tratamento endodôntico é imprescindível que a escolha dos componentes da obturação seja feita com critério em relação à compatibilidade biológica, visando tornar o processo de reparação mais rápido e efetivo. **Objetivo e metodologia:** Este estudo teve o propósito de analisar as reações teciduais apicais e periapicais em pré-molares de cães, após pulpectomia e preparo químico-cirúrgico, cujo limite foi definido em nível do delta apical, e obturação com cones de guta-percha e dois cimentos contendo hidróxido de cálcio – Sealapex® e Apexit®. Foram analisados os eventos histopatológicos em períodos de 7, 21 e 45 dias, a fim de comparar os fenômenos de inflamação e de reparação. Decorridos os períodos de observação, os cães foram sacrificados, e as peças, processadas para análise histológica. **Resultados:** Empregando-se análise quantitativa-qualitativa, verificou-se que o cimento Sealapex® promoveu inflamação entre discreta e moderada em todos os períodos, que evoluiu para reparação aos 45 dias. Houve maior dispersão do cimento Apexit® no tecido periapical, que promoveu intensa atividade macrofágica na área, ampliando os fenômenos de inflamação, mesmo aos 45 dias. **Conclusão:** Ambos os cimentos provocaram necrose em algumas ramificações do delta apical, ocorrendo porém em maior número nos dentes obturados com Apexit®. Da comparação entre o emprego dos dois cimentos, o Sealapex® foi o que apresentou melhor tolerância tecidual e, por consequência, evolução de reparação mais favorável.

## **Introduction**

Upon the end of the endodontic treatment, the tooth's clinical and radiographic follow-up and its adjacent areas become a major concern, which should be interpreted by monitoring the tissue reactions that occur on the periapical area, and the evolution of reparative process (Huang *et al.*, 2002 [12]).

The tissue reparative mechanisms surround the proliferation and organization of the healthy conjunctive tissue, which could be influenced by factors of local or systemic orders.

In pulpotomies, the preservation of the apical stump vitality has been considered relevant for repair, while in cases of pulp necrosis, the growth of the periodontal ligament of the conjunctive tissue into the

inside portion of the apical root canal is expected (Holland *et al.*, 1999 [11]).

The reparative process evolution confirmation, obtained by means of radiographic control, indicates a satisfactory treatment and, most likely, that the materials used to fill the root canal do not interfere negatively in tissue system, and, with this, the cellular reorganization, which indicates the success of the endodontic treatment (Leonardo *et al.*, 1997 [13]). Previous studies conducted by Tavares *et al.* (1994) [17] demonstrated an acceptable tolerance of the conjunctive tissue to the gutta-percha. Thus, the only doubt remaining would concern the filling cement. In this case, it is important that the cement is characterized by a great tissue tolerance and detains satisfactory physical-chemical properties (Willershausen *et al.*, 2000 [18]).

In order to perform studies which aim the analysis of periapical inflammatory reactions developed by filling material and the tissue reparative process evolution, many authors used laboratory animals as dogs, rats and monkeys (Berbert *et al.*, 2002 [2]; Leonardo *et al.*, 1997 [13]), considering the histopathological events occurred in determined experimental periods. Thus, the objective of this study was to compare the evolution of the apical and periapical reactions of dog's teeth filled with two endodontic sealers: Sealapex and Apexit.

## Material and methods

Twelve dogs, with no specific breed, all males, ages ranging from 12 and 15 months, derived from the kennel of Mogi das Cruzes's University were selected. The project was approved by the UMC Animals Manipulation and Experimentation Ethical Committee (CEMEA-UMC), under the protocol 001/03.

The selected teeth were the two lower premolars from each dog, one from the right side and other from the left side.

The dogs remained 12 hours without being feed before the anesthesia, in order to avoid interference in the medication action. During this period received only *ad libitum* water. During all the surgical procedures, the animals were placed dorsally and horizontally, being submitted to general anesthesia.

A mouth opener was adapted between the upper and lower canine teeth, on the opposite side of the procedures, to facilitate the tooth visualization. The lower premolars, individually treated, were isolated with clamps, rubber dam and Ostby arch, allowing the control of the operation field. A radiographic exam, on a periapical, was initially performed on each tooth, by means of control.

The access to the pulp chamber was performed, proceeding to the irrigation and aspiration with 5 ml of 0,5% sodium hypochlorite - Dakin Liquid -, by canal.

Considering that dog's premolars teeth have two root canals (mesial and distal), the odontometry was performed, in order to obtain the real working length, defined along with de delta apical. The pulpectomy was performed, simultaneously to the instrumentation of the root canals, with K type files, according to a serial conventional technique, utilizing auxiliary chemical substances, Dakin liquid and an Endo-PTC cream, in the following proportion: for each file, the canal was filled with the cream and activated by 0,5 ml of sodium hypochlorite, approximately. On each instrument replacement, the canal was irrigated with another 5 ml of Dakin liquid. As a final irrigation-aspiration, 5 ml of 0,5% sodium hypochlorite were applied, followed by 5 ml of 15 % EDTA-T.

The canals were dried with absorbent paper cones and the filling was performed, with lateral condensation technique, varying the sealer.

The Sealapex (Kerr, USA, Lot 1-1324) was applied in the correspondent premolars to the right lower semi arch, and the Apexit (Vivadent, Germany, Lot B42585) sealer on the left side.

After cutting the exceeding material from the coronary chamber and cleaning it, a vertical condensation of gutta-percha was performed on each canal and cavity was filled with silver amalgam restoration. A final radiography was performed to verify the quality of the filling.

The subjects were randomly allocated into three groups, according to its experimental analysis period, as the following pattern: Group A – seven day period, Group B – twenty one day period and Group C – forty five day period.

After 7, 21 and 45 days, the animals from each period were sacrificed by an intravenous anesthetic overdose. The necropsy was performed by removing, along with the tooth, enough adjacent tissue, which was immediately fixed in 10% formol for further histological analysis. After 24 hours the teeth were prepared for the beginning of the laboratory phase.

The teeth were decalcified and dehydrated in order to be immersed in paraffin. The paraffin blocks, previously identified, were submitted to serial cuts with a thickness of 6 micrometers, assembled on glass laminas e stained by HE Technique (Hematoxylin and Eosin) and Gomori trichrome.

The analysis of the results was performed by light microscopy, considering the tissue responses caused by both sealers. The assessment was determined by following the protocol proposed by Leonardo *et al.* (1997) [13], marking the respective apical and periapical histopathological events on degrees of intensity, as follows:

**0** – Non significant (NS);

**0 to 1** – between non significant and discrete (NS/D);

**1** – Discrete (D);

**1 to 2** – between discrete and moderate (D/M);

**2** – Moderate (M);

**2 to 3** – between moderate and intense (M/I);

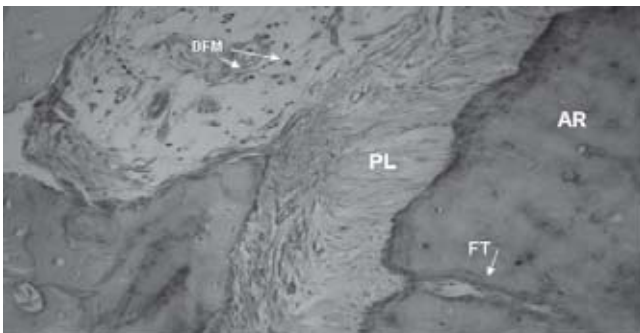
**3** – Intense (I).

The scores were used to record the inflammatory infiltrate, with its cellular types, divided into polymorphonuclear neutrophils cells, mononuclear and inflammatory giant cells, which arithmetical mean resulted on in the inflammatory general index. On the other hand, the general index for the collagen evolution was defined by the tissue regeneration capacity or to its capacity to decrease the existing inflammation process.

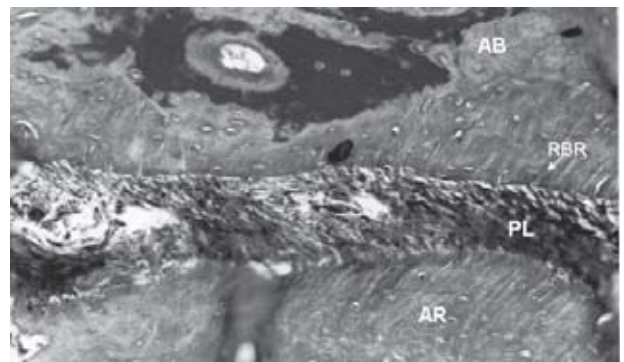
## Results

The histopathological events showed an inflammatory infiltrate with predominance of mononuclear cells in every period with both sealers, presenting decreasing values of polymorphonuclear neutrophils in relation to the increase of time analysis and the absence of giant inflammatory cells. The Sealapex showed the lowest inflammatory general scores, characterizing a discrete/moderate reaction in the periods of 7 to 21 days and a non significant/ discrete in the 45 days period.

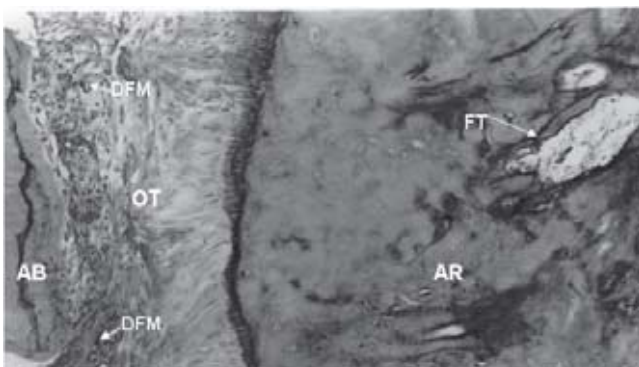
In group with the Sealapex sealer at 7 days it was verified a small thickening of the periodontal ligament, bone resorption with a small activity and some disperse particles of sealer in the foraminas and in the periapical tissue (figure 1). At Group B (21 days) showed a smaller resorption and grater tissue organization (figure 2). At 45 days, the delta apical presented a great amount of foraminas filled by tissue and the periapical tissue (figure 3). It was noted some disperse material in the three periods of analysis, however, without interfering in the decrease of the inflammatory reaction.



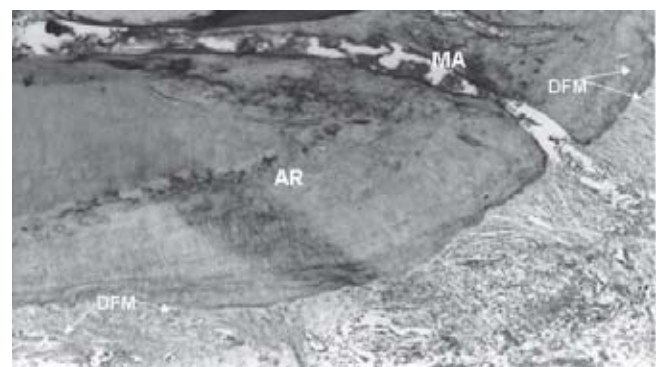
**Figure 1** - Sealapex - 7 days - Group A - Neoformed tissue (TF) in the apical foramen region (AR), disperse filling material (DFM) in the periapical region and active bone reabsorption. Original enhance 200x, HE



**Figure 2** - Sealapex - 21 days - Group B - Thickened periodontal ligament (PL). Alveolar bone (AB) in reparation stage (RBR). Original enhance 400x, Gomori trichrome

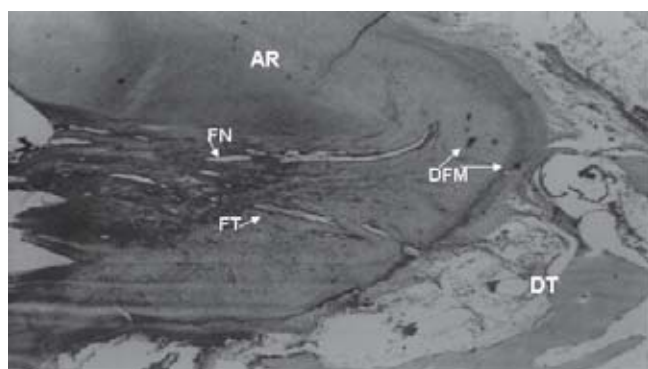


**Figure 3** - Sealapex - 45 days - Group C - Ramification region of the delta apical (RA), with disperse filling material (DFM) in the periapical region. Organized periodontal tissue (OT). Alveolar bone region (AB). Original enhance 400x, HE

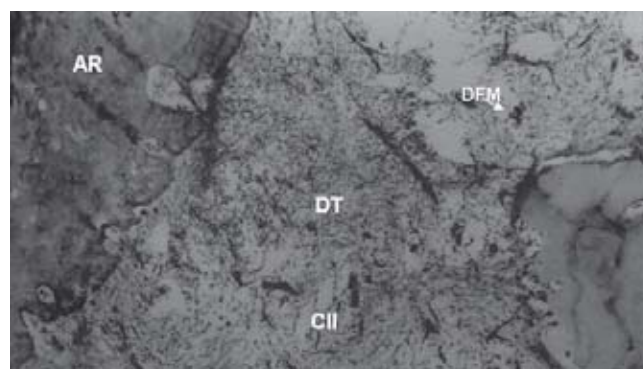


**Figure 4** - Apexit - 7 days - Group A - Residual material in the foramina (DFM), causing intense macrophagic activity (MA) and beginning of local tissue necrosis. Original enhance 400x, HE

The teeth filled with Apexit sealer, it was verified a more intense inflammatory reaction, with greater amount of disperse material when compared with Sealapex, leading to the increase in the inflammatory general scores in the three periods (figure 4). Likewise, there was an active bone resorption and the periapical tissue was extremely unorganized at the 45<sup>th</sup> day, with an increase in the inflammatory index and evolution of the slowest collagen growth (figures 5 and 6).



**Figure 5** - Apexit - 21 days - Group B - Delta apical's ramifications filled or not by tissue (FN/ FT). Presence of disperse filling material (DFM). Disorder of periodontal apical tissue (DT). Original enhance 100x, HE



**Figure 6** - Apexit - 45 days - Group C - Tissue disorder (DT) in the periodontal apical region, characterizes by chronic inflammatory infiltrate (CII). Presence of disperse filling material (DFM). Original enhance 200x, HE

## Discussion

Constituted by many stages, the endodontic treatment is performed with several substances and materials, which allow the disinfection and cleaning of the root canal, maintenance of sanification and its filling. It is during the filling stage of the root canal that the sealers are used, along with gutta-percha, that can be found in many bases and compositions and for that reason, tend to show different physical-chemical and biological properties.

Sealers containing-calcium hydroxide have been widely used, both in clinical and research purposes, due to properties that allow the repair of periapical tissues, as these sealers present elevated pH, which promote the alkalinity of the environment, due to its capacity do give calcium ions to the tissue, which favors the biological sealing of the apical foramen (Foreman e Barnes, 1990 [4]; Holland *et al.*, 1999 [11]; Silva *et al.*, 1997 [15]; Holland *et al.*, 1998 [10]).

*In vitro* and *in vivo* studies have been broadly conducted in order to compare the sealers properties that are release on the market every year (Leonardo *et al.*, 1997 [13]; Holland *et al.*, 1999 [11]; Willershausen *et al.*, 2000 [18]; Berbert *et al.*, 2002 [2]; Huang *et al.*, 2002 [12]).

In this study, two calcium hydroxide sealers were comparatively analyzed – Sealapex and Apexit – considering the apical and periapical tissue reactions in dog's teeth, which occurred in three experimental periods. Some discrepancies are observed among the conclusions of studies performed *in vitro* and *in vivo*, with the same sealers, which do not provide enough support for their choice, as presented by Beltes *et al.* (1998) [1], Huang *et al.* (2002) [12], Leonardo *et al.* (1997) [13] and Friedman *et al.*, 1997 [5].

The endodontic treatment of the dog's premolars was performed in one session, justified by the fact of being cases of living pulp (Camps *et al.*, 1992 [3]; Soares *et al.*, 1990 [16]; Holland *et al.*, 1990 [7]; Holland *et al.*, 1994 [8]; Leonardo *et al.* (1997) [13]. The real length of work was determined in the delta apical limit, present in every root canal of the dogs, in agreement with the studies of Holland and Souza (1985) [6]. Studies like this show that the comparison among the apical sealers occurred in cases of forced foramina opening and when the limit was determined beyond the delta apical, the latter allowed a major mineralized tissue disposition in the foramen, which corroborates the choice of this study in determining the work length on a level already described.

The peculiarity of this study of using the delta apical as an instrumentation limit determined the main difference found in the studies of the literature, as most of them were performed by forcing the opening of the foramina, with the objective of analyzing the inflammatory histological events only on the supporting tissue. Another factor that corroborates this choice, is the supposition that, being the delta apical a group of foraminas that present a fan-like shape in the apical region of the dog's teeth, as a result, the tested material remained away from this region, not interfering on the tissue repair, as long as there was no contact between the tested material and the supporting tissue. This study proves that filling material tends to move from the apical to the periapical region, as in the foraminas is possible to find sealer particles that change the initial situation, leading to a mild inflammation.

The analysis time of 7, 21 and 45 days were determined to allow the follow-up of the repairing tissue evolution, as in the study of Nassri *et al.* (2003) [14]. The determination of short experimental times

was possible by the work limit established away from the radiographic apex, which did not cause great damage to the periapical region, and consequently, did not affect the results. This fact is in agreement with the studies of Holland *et al.* (1990) [7], and Berbert *et al.* (2002) [2], who performed the opening of foramina and had their analysis time extended.

A chart was used to record the results, following the pattern of most studies in this area. To create this chart, the model considered was from Leonardo *et al.* (1997) [13]. The analysis was performed on histological cuts by a light microscope, with the score records, according to the same authors.

The results show a chronic inflammatory infiltrate, with predominance of mononuclear cells, followed by a small number of polymorphonuclear neutrophils, using both sealers, in every experimental group. The chart of inflammatory events found on the Sealapex sealer shows similarities to those found in the literature, with a decrease in the inflammatory general indexes at 21 and 45 days (Holland *et al.*, 1990 [7]; Berbert *et al.*, 2002 [2]).

It was possible to observe, on most ramifications of the delta apical, conjunctive tissue without necrosis, mainly on the teeth sealed with Sealapex, with the record of apical partial biological sealing, in agreement with the studies of Holland and Souza (1985) [6]. However, Leonardo *et al.* (1997) [13] described a poor performance of the Sealapex sealer, also on dog's teeth. In opposition to such observation, fact is, as the experimental period increased, the inflammation index decreased, and there was a greater evolution in the collagen growth.

On the other hand, the particles of Apexit showed greater dispersion on the periapical tissue, including the 45 days period, maintaining the inflammation index between discrete and moderate, making the evolution of repair more difficult. The ramification with tissue necrosis was observed and mononuclear phagocytes were found far from the apical foramen, causing tissue disorder that persisted until 45 days. These findings agree with previous research of Leonardo *et al.* (1997) [13] and Nassri *et al.* (2003) [14]. *In vitro* studies disagree with the potential irritating effect of Apexit sealer, showing better results in cellular cultures when compared with other sealers, including Sealapex (Beltes *et al.*, 1998 [1]; Huang *et al.*, 2002 [12]).

The presence of apical biological sealing shows that the sealers interfere significantly on the neoformed tissue deposition on the apical region

of the teeth. According to Holland *et al.* (1990) [7], this is due to the stimulation repair generated by the deposition on hard tissue by the calcium hydroxide.

On a general context, the Sealapex sealer allowed a better repair evolution, with inflammation index tending to decrease according to the experimental period, in opposition to the Apexit sealer, that maintained the inflammatory reaction stabilized between discrete and moderate in every group. Statistically, the samples analyzed at 45 days, for both sealers, showed significant differences.

It is important to notice, based on the results, that the correlation between the inflammatory index and the evolution of the collagen growth exists and it is confirmed by the statistical test of Spearman. The conjunctive tissue is not able to repair itself in the presence of great quantity of inflammatory cells (Holland *et al.*, 1998 [9]).

## Conclusion

Based on the data obtained from the histopathological events analyzed on the periods proposed in this study, it is possible to conclude that both endodontic cements tested – Sealapex and Apexit – showed to be irritants to the apical and periapical tissue of the dog's teeth, however, the Sealapex provided lower tissue inflammatory indexes during the experimental periods, which allowed greater repairing evolution, when compared with Apexit sealer, which presented greater dispersion.

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