PULP RESPONSE TO BERIPLAST, GELFIX AND CALCIUM HYDROXIDE IN PULPOTOMIZED TEETH OF DOGS: A HISTOPATHOLOGICAL STUDY

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Abstract
The aim of this research was to histologically evaluate the pulp dressing potentials of two commercial surgical materials (Beriplast and Gelfix) and compare with that of calcium hydroxide after experimental pulpotomy in dogs. Following the standard pulpotomy and wound treatment procedure in 60 mature molar teeth in five dogs, dressing materials were placed over the pulp stumps and then the cavities were restored with IRM and amalgam. After 7, 30, 60 and 90 days, the tissues were collected. Histological sections were prepared and examined for inflammation, degenerative changes and bridge-like and tertiary dentine formations. The results showed that calcium hydroxide exhibited a more favourable pulpal response profile than the experimental pulp dressing materials, Beriplast and Gelfix, in pulpotomized dog teeth.

Key words: calcium hydroxide, pulp, histopathologic study.

INTRODUCTION
A number of pulp dressing agents have been investigated in regard to their potential induction of pulp healing and hard tissue barrier formation following pulp amputation (1,7,17,32,41). The healthy pulp has a good healing potential when it is exposed (13). During pulpotomy, the surgical removal of the coronal pulp causes injury and to use a dressing material that would lead to healing and allow for the continuity of the normal pulp physiology, would be ideal (7). Collagen, a biomaterial, was evaluated as a pulp dressing agent in several studies, with conflicting results (1,5,7,8,10,17,19). However its regulating role in development and tissue repair is well known (24). And, adhesives have been recommended by several authors for wound closure, haemostasis and promotion of wound healing in oral and maxillofacial surgery (15,16,22). Beriplast, a fibrin glue product, has been used successfully to attain haemostasis in patients on anticoagulation therapy (14), and in patients with bleeding disorders undergoing tooth extraction (23). Therefore the purpose of this study was to evaluate two biological materials widely used in surgical procedures, a fibrin seal (Beriplast P) and a collagen based sponge (Gelfix) as pulp dressing agents in pulpotomy.
zed dog teeth and to compare their potential use with that of calcium hydroxide, an established material.

MATERIALS AND METHODS
A total of 60 mature permanent molar teeth from five young dogs, 3 to 4 years old, were used in this study. After premedication with Xylazine (2 mg per kg body weight, Rompun-Bayer; Germany), anesthesia was induced (i.v. injection of Ketamine, 5 mg per kg body weight, Ketalar-Park Davis, USA) and maintained under 3% Halothane (Hoechst, Germany) following tracheal intubation. After the teeth were thoroughly scrubbed with pumice and gauze soaked in 4% H2O2 solution, they were isolated with rubber dam. The operation site was cleaned with 5% tincture of iodine. Pulp exposure was created with a sterile round diamond bur, at high speed, under adequate water spray. After removing the roof of the pulp chamber, the coronal pulp was amputated at the orifices of the root canal walls, by means of a sterile long-neck round bur operated at low speed followed by saline rinses. Haemorrhage was arrested with sterile cotton pellets. One of the following dressings were then immediately applied over the pulp stumps: a lyophilized bovine Achilles tendon collagen sponge (Gelfix, Eurosearch, Italy), a commercial fibrin seal (Beriplast, Behringwerke, Germany) and calcium hydroxide – normal saline paste (Calcium hydroxide, Sultan, USA). All cavities on both upper and lower molars on one side were dressed with calcium hydroxide, and the ones on the other side with Beriplast or Gelfix. The teeth were then sealed with IRM and the remainder of the cavities were restored with amalgam. The animals were sacrificed and the tissues were collected at 7, 30, 60 and 90 days, fixed in 10% formic acid. Following paraffin embedding, serial 8 micron sections were cut in the bucco-lingual plane and stain with hematoxylin and eosin, or Brown and Brenn bacteria staining method (34). Unfortunately, all of the five teeth in seven-day Beriplast group were lost during the processing for histologic sectioning. The microscopical evaluation was performed according to the following modification of the widely used differential diagnosis scoring criteria (2,3,8,11,30). Inflammatory cell infiltration:
1- No or sparse (slight) leucocytes in contact with the original wound
2- Localised (moderate) leucocyte infiltration subjacent to, or some distance from the original wound
3- Massive (severe) leucocyte infiltration throughout the pulp

Degenerative changes some distance from the

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RESULTS

The scores for the selected criteria are given in Table 1. Calcium hydroxide (Day 7): All of the teeth were free of inflammation. Overlapping grades of degenerative changes of pulpal tissue were observed in all specimens, and these changes were as fibrin core organization in 3 teeth (Figure 1) and as collapsed pulp zones in the remaining 2 teeth. All teeth showed the presence of an incomplete bridge-like fibrous matrix formation at the canal orifice (Figure 1) and this matrix formation was adjacent to the dressing material in 3 specimens, whereas in the other 2 specimens, it was at the

some distance from the dressing. No tertiary dentine formation was observed in any of the teeth.

Calcium hydroxide (Day 30): Moderate inflammatory response with neutrophil leucocytes and few lymphocytes were detected in 2 specimens (Grade 2) (Figure 2a), however in the remaining 3 specimens, sa-
calcium hydroxide (Day 60): Moderate inflammation was seen in only one specimen. Degenerative changes were found characterised by fibrin core organisation in 2 teeth (Grade 2) (Figure 3a) and by liquefaction necrosis in 3 teeth. New hard tissue formation in 2 teeth completely bridging the canal orifice (Figure 3b) and incompletely bridging in one tooth was observed adjacent to the dressing material. Heavy tertiary dentine deposition was seen in one tooth coronal to the application site, in another one apically and in the remaining three both coronally and apically (Figure 3a and 3b).

Calcium hydroxide (Day 90): One specimen showed signs of severely inflamed pulp and another one moderately inflamed pulp (Figure 4). Three teeth exhibited coagulation necrosis characterised by fibrin core organisation (Figure 4). Bridge-like dentine formation in front of the canal orifice in complete form in 3 teeth and in incomplete form in 2 teeth was observed adjacent to the dressing material (Figure 4). One tooth coronal to the application site and three teeth both co-
Figure 5. Tooth treated with Beriplast: 30 days. Extensive necrotic areas due to pulp degeneration, tertiary dentine deposition and bridge-like dentine formation (H.E. x 100).

ronally and apically (Figure 4) exhibited tertiary dentine formation.
Beriplast (Day 30): Neutrophile leucocyte and lymphocyte infiltration was found to be moderate in 2 teeth (Grade 2) and severe in one tooth (Grade 3). Coagulation necrosis due to fibrin organisation was detected in 3 specimens and in the remaining 2 specimens, the pulp tissue exhibited extensive necrotic areas due to tissue degenerations (Figure 5). Incomplete bridging of the canal orifice with hard tissue formation was observed in 2 teeth, (Figure 5) and complete bridging in one tooth was detected. A thin line of tertiary dentine deposition was found in 3 teeth coronal to the application site (Figure 5).
Beriplast (Day 60): In only one tooth, severe inflammation was observed. Two teeth exhibited coagulation type of moderate tissue degeneration, whereas the remaining 3 teeth showed more severe degenerative changes. Complete bridge-like dentine formation in one tooth and an incomplete one in one tooth were observed. In 3 teeth apically and in one tooth both coronally and apically, tertiary dentine formation was found.
Beriplast (Day 90): None of the teeth presented inflammation. Coagulation necrosis in one specimen, liquefaction necrosis in 2 specimens and intense tissue degenerations in the remaining 2 specimens were detected. In one tooth, complete and in another one, incomplete hard tissue bridges over the canal orifice were observed. Tertiary dentine formation coronal and

both coronal and apical to the application site was found in one and 2 teeth, respectively.
Gelfix (Day 7): Three specimens exhibited severe infiltration of neutrophile leucocytes, monocytes and
lymphocytes. Degenerative changes in pulpal tissue characterised by coagulation necrosis were detected in 3 teeth, liquefaction necrosis in one tooth and abscess formations in one tooth. No bridge-like or tertiary dentine formation was observed in any of the specimens. Gelfix (Day 30): Only one specimen revealed inflammatory cell infiltration, which was moderate. Coagulation necrosis in one specimen (Figure 6a), liquefaction necrosis in 3 specimens and abscess form degeneration in the last specimen were observed. In only one tooth, hard tissue formation not totally bridging the canal orifice was deducted. Coronal tertiary dentine formation in 2 teeth (Figure 6a) and both coronal and apical tertiary dentine formation in 3 teeth were found.

Gelfix (Day 60): In 2 teeth, severe inflammation was evident (Figure 6b). In 2 teeth, coagulation necrosis and in 2 other teeth, liquefaction necrosis were observed. In the remaining one tooth, a considerable degree of degenerative changes was noted (Figure 6b). Bridge-like dentine formation across the canal orifice in incomplete form was seen in 2 specimens (Figure 6b). In 2 teeth at the canal orifice and in another 2 teeth along the pulpal aspect of radicular dentinal wall, tertiary dentine formation was found (Figure 6b).

Gelfix (Day 90): Severe inflammatory reaction was detected in only one specimen. Liquefaction necrosis was detected in all specimens. Incompletely bridging in one tooth and completely bridging in 2 teeth of the canal orifice by new dentine formation were found. Tertiary dentine formation coronal to the application site was observed in 4 teeth (Figure 6c).

**DISCUSSION**

Pulpal response to calcium hydroxide in dog teeth has been investigated by many authors (8, 21, 35, 39a). Our results support the findings of Tziasfas and Molyvdas (35), who reported that dog pulp responds to calcium hydroxide treatment with a lower success rate of healing with hard tissue barrier formation than human (18, 31) and monkey (9, 33, 41) pulpal tissue. However, our findings showed that overall pulpal response to calcium hydroxide at all observation periods was found to be more favourable, with a higher incidence of bridge-like dentine formation and a heavier tertiary dentine production, than Beriplast and Gelfix.

Schröder (27) reported that the development of a firm necrosis as the result of the chemical injury caused by the hydroxyl ions of calcium hydroxide applied to the exposed pulp, stimulates the tissue to defence and repair by causing slight irritation. It has been stated by Cox et al (4) and Hey s et al (9) that to induce hard tissue repair, a low grade of irritation is needed. However, Hey s et al (9), although infrequently (20% teeth capped 21 days or more) and apparently due to the extension of the mineralised tissue from the lateral walls, found mineralised tissue formation across the exposure site in primate teeth treated with Teflon, and they suggested that the distribution of mineralised tissue formation might be due to the morphogenetic protein that diffuses from the intact dentinal and predentinal surfaces and/or to the dentine chips which could signal progenitor cells to differentiate into odontoblast-like cells (25, 37, 40), and important differences in the healing sequences responsible for the development of the newly differentiated functioning odontoblasts may be represented by the differences in inflammatory responses caused by the initial injury and treatment with calcium hydroxide, which results in superficial necrosis, and with Teflon, an inert material. Ogumbe et al. (20) have demonstrated that in repairing dental pulps, under both inert and bioactive materials, cell differentiation and reparative dentine bridge formation occur and they have suggested that components of the extracellular matrix may be more important than the pulp capping agents for this reaction and the signal for the differentiation of dentin forming cells may be intrinsic.

Tziasfas et al (36), in a comprehensive review, have stated that "dentine matrix should not be considered as an inert dental hard-tissue, but rather as a potential tissue store of bio-active molecules (particularly growth factors) waiting to be released if appropriate tissue conditions prevail".

The mechanism involved in the induction of hard tissue formation after treatment of pulpotomized dog teeth with the biomaterials, Beriplast and Gelfix (a collagen sponge), in the present study is not fully understo-
od. It has been shown that extra-pulpal blood clot impairs healing and reduces the incidence of dentine bridge formation following capping with calcium hydroxide (26). Since the experimental materials used in our study are haemostatic agents, they could have contributed to the hard tissue formation process by preventing excess clot formation, which has a chemotactic effect on inflammatory cells (26). Moreover, in addition to its known haemostatic properties, collagen fibers provide extracellular support for tissues (17) and influence mineralisation by orienting hydroxyapatite crystals (5). The findings of Bimstein and Shoshan (1) demonstrated the healing-promoting effect of an enriched collagen solution and the regeneration of the dog pulp tissue following pulpotomy, as a result of chemotactic attraction of fibroblasts by the collagen gel.

Favourable responses to an enriched collagen solution with proliferation of connective tissue cells and blood vessels coronal to the newly formed bridges were observed in pulpotomized teeth of dogs by Bimstein and Shoshan (1) and of monkeys by Fuks et al (6). After direct pulp capping on inflamed dental pulp wounds of baboon teeth, Oguntebi et al (19) found greater amount of irritation dentine production in teeth treated with calf skin collagen and indomethacin than in those treated with zinc oxide-eugenol, although an inflammatory response to both materials, especially to collagen, was present from the outset and became progressively more severe after 90 days. Nevins et al (17) reported that a collagen gel used in pulpotomy and partial pulpectomy procedures in monkey teeth is resorbed and replaced by vascularized cellular hard tissue resembling dentine or cementum. The finding of tertiary dentine and, although not consistent, bridge-like dentine formation in teeth treated with Gelfix in the present study, is in agreement with these previous reports (1, 6, 17, 19).

However, in one of our previous studies (8) in dog teeth treated with the same experimental materials, Beriplast and Gelfix, by direct pulp capping procedure, no hard tissue production, neither bridge-like nor tertiary dentine formation, could be detected and a chronic inflammatory response with extensive pulp tissue degenerations had persisted in all specimens even at later observation periods. One may speculate whether the difference in the results of our two studies may be attributed to the resultant irritation from bacterial microleakage, which could be eliminated by the thicker IRM and amalgam restorations in pulpotomy but not in direct pulp capping procedure, or which occurred as a result of the surface seal in the latter procedure. But the common finding in both studies was that calcium hydroxide exhibited more favourable and consistent responses than Beriplast and Gelfix.

Persistent chronic inflammation and pulp tissue degenerations in some teeth treated with each of the material used in this study could be partly attributed to the thinness of the radicular pulps in dog molar teeth and to the further narrowing of the pulpal space because of new hard tissue formation, probably causing circulatory disturbances. In addition, the results of previous studies on dog pulp have shown less favourable responses to calcium hydroxide application than in human (18) and rat teeth (39). One contributing factor to these findings could also be related to the inconvenience of applying Beriplast and Gelfix because of their being translucent and non-setting materials, resulting in inadvertent pushing of these materials during placement and further cavity restoration into and compression of the residual pulp tissue. Altogether these factors along with operative difficulties in the posterior region could have played a role in our results by delaying the healing process. A 90-day observation period seems to be insufficient and, presumably, greater healing might be expected over a longer period of time. Therefore, histologic evaluations with a longer period of postoperative time would probably better reveal the real success rate.

Dentine bridge formation has being a success criterion, but it is sometimes associated with chronic inflammation, microabcesses and pulpal necrosis (12, 28, 29) and not accepted as a complete seal for the injured pulp (12, 39), is generally regarded as a valid indicator of pulp healing (20, 36) by demonstrating the continued function of the delicate cells, odontoblasts (39), which in turn reveals the state of the pulp (38). The presence
of the newly formed tubular dentine is another demonstration of the same phenomenon (39). In our study, we have observed considerable tertiary dentine deposition and some bridge-like dentine formation during the observation period in teeth treated with Beriplast and Gelfix, however, mostly in association with the degenerative changes of the remaining pulp tissue. Therefore, we believe that the data generated from this study should be interpreted with caution and is not considered to be clinically significant.

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Özet

Bu çalışmamın amacı, pulpa kapama potansiyeline sahip iki ticari ürünü (Beriplast ve Gelfix), köpeklerde pulpatomiden sonra histoloji olarak değerlendirilmek ve kalısyum hidroksitinde karışımların, beş köpeğin gelişmiş 60 molar dişinde, standart pulpatomi ve yara tedavi işlemlerini takiben, pulpa kapama materyalleri kalan pulpa bütünü nın üzerine yerleştirildi ve daba sören kaviteler IRM ve amalgaama restore edildi. 7, 30, ve 90 gün sonra dokular toplandı. Histoloji kesitler hazırlandı ve infiltrasyon, dejenerasif değişiklikler, köprü henset ve testiyer dentin oluşumları incelendi. Sonuçlar, pulpatomize yapımış köpek dişlerinde, kalısyum hidroksitindeki nedenler pulpa kapama materyalleri Beriplast ve Gelfix'den daba olumlu bir pulpa cevabı sergilediği gösterdi.

REFERENCES


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